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Benomyl/Thiophanate-Methyl

Position Document 4

Special Pesticide Review Division

Office of Pesticide Programs

Office of Pesticides and Toxic Substances

U.S. Environmental Protection Agency

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Executive Summary

In December, 1977, the U.S. Environmental Protection Agency (EPA) issued a Rebuttable Presumption Against Registration (RPAR) for fungicidal products containing benomyl (Methyl-1(butylcarbamoyl)-2-benzimidazolecarbamate). This was based upon the ability of benomyl or its metabolite, methyl 2-benzimidazole carbamate (MBC), to cause teratogenic, mutagenic and spermatogenic effects. Its potential to cause hazards to nontarget organisms and aquatic organisms was also considered. In August 1979, the EPA issued a proposed regulatory decision for products containing benomyl. That decision proposed to require the use of cloth masks for persons engaged in mixing/loading for aerial application of benomyl. It also proposed to require aquatic monitoring of benomyl residues to assess further the risks to aquatic organisms. In addition, the EPA issued an RPAR against the fungicide thiophanate-methyl^{1/} in December, 1977 because its metabolite, MBC, had the potential to cause mutagenic effects and because thiophanate-methyl had the potential to cause adverse effects to nontarget organisms. On October 3, 1979, EPA published a notice proposing to conclude the thiophanate-methyl RPAR because significant risk could not be shown to occur from its use.

Subsequent to publication of EPA's proposed regulatory decision, new data were received from duPont indicating that benomyl and its metabolite, MBC, were hepatocarcinogens in mice. In addition, evidence for an increased incidence of lung tumors was shown in the study received for benomyl. Additional data were also received on exposure, mutagenicity, oncogenicity and spermatogenic effects. The EPA revised its risk assessment based upon this new information and has determined the following.

Benomyl and the MBC metabolite of benomyl and thiophanate - methyl are capable of causing effects to the cellular spindle apparatus. The impact of this effect to human health cannot be adequately assessed at this time. Possible mutagenic risk alone will not lead to a recommendation for regulatory action now. However, the data on the mutagenic potential are supportive of the qualitative determination of benomyl's teratogenic, spermatogenic and oncogenic risks and thiophanate-methyl's oncogenic risks.

Benomyl has the potential to cause teratogenic effects. The NOEL for these effects is tentatively set at 30 mg/kg/day based on the results of a gavage study in rats. DuPont is now performing tests to confirm this level. A number of dietary studies of teratogenicity have reported negative results. Margins of Safety (MOS - estimated exposure/no observed effect level) for teratogenicity from worst-case dietary exposure range from 254 to 600,000 (single serving basis). For workers/users they range from 86 to dietary background levels without respiratory protection and from 600 to dietary background levels, if a dust mask is used.

Benomyl's potential to cause spermatogenic effects is shown by tests using various dosing routes. The NOEL for these effects is 7.5 mg/kg/day based on an inhalation study in rats. MOS's for workers/users range from 21 to dietary background levels without respiratory protection and from 150 to dietary background levels if a dust mask is used. Margins of safety for dietary exposure range from 64 to 150,000 for worst-case estimates.

Benomyl and MBC, the metabolite of both benomyl and thiophanate-methyl, were shown to be hepatocarcinogens in tests with mice. Based upon the most sensitive parameter for oncogenicity, liver tumors, the upper limit to the oncogenic risk to the general public via dietary exposure was estimated to be about 7×10^{-5} . This exposure was based upon an assumption that residues would be in the diet at tolerance levels. The oncogenic risk based upon average residues is less than 7×10^{-6} . For workers/users the oncogenic risk ranges from $<5 \times 10^{-7}$ to $<3 \times 10^{-5}$ without respiratory protection and from $<7 \times 10^{-7}$ to $<5 \times 10^{-6}$ if a dust mask is used. The risk from thiophanate methyl would not likely be greater than that from benomyl due to their similar uses and metabolism.

The Agency's brief review of the benefits of benomyl in selected crops indicated that the use of alternative control methods would increase production costs from \$26 to \$52 million in a typical year without benomyl. Additional losses for decreased production and higher consumer price impacts would also be expected. The benefits of thiophanate-methyl were not quantified. However, if it is assumed that thiophanate methyl is equally as efficacious as benomyl then it would share in the large combined benefits of both products according to its ability to be market competitive with benomyl.

The EPA will require the use of a dust mask by persons during mixing and loading of benomyl for aerial application. Dermal penetration of benomyl is slight; therefore, the dermal component of exposure has been discounted in the EPA risk estimate. Dust masks are estimated to decrease respiratory exposure by as much as 90 percent, therefore, this requirement will reduce the remaining work-related component of exposure significantly.

The EPA will require a monitoring study of benomyl residues in water from treated rice fields under authority of FIFRA Section 3(c)(2)(B).

^{1/} Benomyl is a fungicide registered for use on various stone fruits, citrus fruits, grapes, mushrooms, berries, apples, pears, rice, tomatoes, celery, mangoes, beans, avocados, cucurbits, peanuts, soybeans, sugar cane, nuts, seeds of various vegetable crops, turf, flowers, and ornamentals. Thiophanate-methyl is registered for use on stone fruits, apples, strawberries, celery, beans, peanuts, soybeans, sugar cane, nuts, turf and ornamentals.

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William Burnam, Ph.D., Deputy Branch Chief, Toxicology Branch, HED, OPP
Christine Chaisson Ph.D., Section Head, Toxicology Branch, HED, OPP
Harry Gaede, Ph.D., Section Head, Economics Analysis Branch, BFS, OPP
Roger Gardener, Toxicologist, Toxicology Branch, HED, OPP
Margaret Jones, Project Manager, SPRD, OPP
Bertram Litt, Ph.D., Statistician, Toxicology Branch, HED, OPP
Tom Miller, Section Head, SPRD, OPP
Ester Saito, Environmental Chemist, Environmental Fate Branch, HED, OPP
Bernard Smale, Ph.D., Section Head, Plant Sciences Branch, BFS, OPP
Minnie Sochard, Ph.D., Toxicologist, Toxicology Branch, HED, OPP

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I. Introduction

A. Legislative Authority

Section 12 (a)(1)(A) of the Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA, (7 U.S.C. 136 et seq.) prohibits the sale or distribution of pesticide products which are not registered by the EPA Administrator, and Section 3 of the Act sets forth the registration procedures. Before a pesticide may be registered, however, the Administrator must determine that its use will not result in "unreasonable adverse effects on the environment," [Sec. 3(c)(5)(C)], defined in Section 2(bb) of FIFRA as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide." Therefore, any decision on pesticide registration must take into account both the risks and benefits associated with the pesticide's use.

Under Section 6(b) of FIFRA, the Administrator may issue a notice of intent to cancel the registration of a pesticide or to change its classification if it appears that the pesticide or its labeling "does not comply with the provisions of (FIFRA) or, when used in accordance with widespread and commonly recognized practice, generally causes unreasonable adverse effects on the environment." Thus, the Administrator may cancel the registration of a pesticide that no longer satisfies the statutory standard for registration. The Administrator may also change the classification of any use of a pesticide if he determines that such a change "is necessary to prevent unreasonable adverse effects on the environment" [FIFRA 3(d)(2)].

To implement its authorized functions, the Agency designed the Rebuttable Presumption Against Registration (RPAR) process to gather data on the risks and benefits associated with the uses of suspect pesticides. By allowing all interested parties to participate by submitting information, the process enables EPA to make balanced decisions concerning problem pesticides. The RPAR process is set forth in 40 CFR 162.11 which describes various risk criteria and provides that an RPAR shall arise if the Agency determines that any of these criteria has been met or exceeded.

Once a rebuttable presumption has arisen, registrants, applicants, and interested persons may submit evidence in rebuttal, or in support, of the presumption. These parties may also submit evidence on the economic, social, and environmental benefits of any use of the pesticide. If the presumptions of risk are not rebutted, the evidence pertaining to benefits must be evaluated and considered together with the evidence pertaining to risk. Various risk-reduction measures and their costs are analyzed. The Agency then determines whether the pesticide may be regulated so that a balance is achieved between risks and benefits. If the statutory balance cannot be reached for any given use, the registrations for that use must be cancelled.

B. Benomyl Background

On December 6, 1977, the Agency issued a Rebuttable Presumption Against Registration and Continued Registration of pesticide products containing benomyl in the Federal Register (US EPA, 1977a). A Position Document (PD 1) on benomyl was published together with the RPAR notice and explained the background and supporting data for the presumption of risk cited in the RPAR notice. Benomyl is a fungicide registered for use on various stone fruits, citrus fruits, grapes, mushrooms, berries, apples, pears, rice, tomatoes, celery, mangoes, beans, avocados, cucurbits, peanuts, soybeans, sugar cane, nuts, seeds of various vegetable crops, turf, roses, flowers, shade trees, and conifers.

In that PD, the Agency presumed against all registrations of benomyl based on its ability, and the ability of its metabolite (methyl 2-benzimidazole carbamate, MBC) to induce mutagenic effects and to reduce spermatogenic activity. Registrations of products intended for home use were presumed against based on teratogenic effects; those for outdoor use were presumed against based upon evidence of reduction to populations of non-target organisms (earthworms); and those intended for direct application to water were presumed against based upon evidence of acutely toxic effects to aquatic organisms (catfish). Concern about N-nitrosamine compound formation from the reaction of MBC with nitrites was also expressed and the Agency solicited evidence bearing on the likelihood and significance of such a reaction.

On August 30, 1979, the Agency published a preliminary notice of determination and notice of availability of the PD 2/3 concerning benomyl (US EPA, 1977c). In that PD 2/3, the Agency determined that the presumptions of mutagenicity (as a spindle poison), teratogenicity, reduction in spermatogenic activity, and acute toxicity to aquatic organisms (catfish) were not rebutted. The presumption against benomyl on the basis of point mutation was rebutted, as was the presumption based on significant local reductions in non-target populations (earthworms). PD 2/3 also presented the Agency's evaluation of the rebuttals it received in response to the original RPAR notice, the Agency's analysis of the risks and the benefits associated with the uses of benomyl, and a general discussion of benomyl use information. It was assumed that use of benomyl adequately demonstrated its benefits at the level of risk then identified.

The PD 2/3 indicated that the risks associated with benomyl use would exceed its benefits unless risk reduction was accomplished by modifications in the terms or conditions of registration. These conditions were as follows:

- (1) require new label warnings on birth defects and reduction of sperm production (five pound bags or larger);
- (2) require water soluble packaging (five pound bags or larger); and
- (3) require use of a cloth mask during mixing/loading for aerial application (five pound bags or larger).

Additional gene mutation studies were proposed to support all uses. A field monitoring study of benomyl residues in water was also proposed.

C. Thiophanate-Methyl Background

On December 7, 1977, the Agency published a Federal Register notice (US EPA, 1977b) and PD 1 announcing the RPAR of pesticide products containing thiophanate-methyl. Thiophanate-methyl is a fungicide registered for use on various stone fruits, apples, strawberries, celery, beans, peanuts, soybeans, sugar cane, nuts, turf and ornamentals. PD 1 described results of tests which showed that thiophanate-methyl is metabolized or degraded to MBC which was considered to produce somatic chromosomal abnormalities and point (gene) mutations in microbial and mammalian systems. The Agency, therefore, presumed against thiophanate-methyl based on evidence of mutagenic effects. In addition, the Agency presumed against thiophanate-methyl based upon evidence that it caused significant local population reductions in non-target organisms (earthworms). Further, as with benomyl, concern was expressed about the potential of MBC to react with nitrites to form N-nitrosamine compounds. The Agency sought additional information on formation of N-nitrosamine compounds, additional teratology information, and information on exposure to thiophanate-methyl.

On October 3, 1979 the Agency published a preliminary notice of determination concluding the RPAR of pesticide products containing thiophanate-methyl and a notice of availability of PD 2 (US EPA, 1979d).

In regard to point mutations, the PD 2 indicated that the available evidence did not clearly demonstrate a risk to humans on uses registered at that time (ornamentals, turf and stone fruits only) and therefore the presumption was successfully rebutted. However, due to the limited data base and equivocal results, the PD 2 indicated that the Agency would be requesting additional studies on thiophanate-methyl's point mutation potential.

In regard to spindle effects the PD 2 indicated that there was some basis for a qualitative presumption against thiophanate-methyl as a result of its metabolite, MBC, initiating potentially heritable effects. However, definitive evidence that MBC induced such effects in germ cells was lacking, though preliminary information indicated that MBC might produce effects leading to chromosomal non-disjunction in germ cells. On that basis the PD 2 indicated that humans exposed to extremely high levels of thiophanate-methyl might be at risk from induction of spindle effects caused by its metabolite MBC. The PD 2 further stated that this presumption had been rebutted since data did not indicate significant risk from estimated exposure levels which were considered to be extremely low. The Agency did indicate that due to the scientific issues regarding thiophanate-methyl's mutagenicity, that its preliminary decision to conclude this RPAR would be submitted to the FIFRA SAP for review.

The PD 2 indicated that the presumption of significant local population reduction in non-target organisms was rebutted since the toxic effects were limited to the site of application, the impact of earthworm loss did not extend to adjacent areas, the populations could rebound to normal within a few years after termination of thiophanate-methyl treatments, and since the sites of application were reasonably limited.

D. External Review

Sections 6(b) and 25(d) of FIFRA require that the Agency submit notices of the proposed decision to the Secretary of the U.S. Department of Agriculture (USDA) for comment on the impact of the proposed action on the agricultural economy and to the FIFRA Scientific Advisory Panel (SAP) for comment on the impact of the proposed action on health and the environment. In accordance with FIFRA, the Secretary and SAP were invited to comment in writing within 30 days of receiving the benomyl notice. Though not a statutory requirement, the Agency also submitted the preliminary notice concluding the thiophanate-methyl RPAR to the SAP for review because of the scientific issues raised in the mutagenicity presumption. The Agency is required to publish their written comments, if submitted within 30 days of the receipt of the Notice, and the EPA Administrator's response to these comments.

Although not required to do so under the statute, the Agency decided that it was consistent with the purposes of the RPAR process and the Agency's overall policy of open decisionmaking to also afford registrants and other interested persons an opportunity to comment on the basis for the proposed action while it was under review by the Secretary of Agriculture and the SAP. The benomyl and thiophanate-methyl Position Documents were therefore made available to all interested parties for comment.

This Position Document 4 concludes the RPAR process for benomyl and thiophanate-methyl. The RPAR of each of these chemicals was initiated at about the same time since both chemicals have the same degradation product, MBC, which is the fungitoxic moiety. Benomyl and thiophanate-methyl are also registered for use on many of the same commodities.

Since the preliminary notices of determination and availability of the Benomyl PD 2/3 and the Thiophanate-Methyl PD 2 were published (August 30, 1979 and October 3, 1979, respectively), the Agency has received new information and a number of comments from interested parties. In addition, new uses of thiophanate-methyl have been registered since that time necessitating consideration of the risk from an expanded set of uses instead of the few that were previously registered. The new information, including the results of additional mutagenicity, teratogenicity and chronic feeding studies on benomyl and MBC submitted by DuPont, resulted in revisions in the risk analyses; this is presented in detail in Section II of this document. A qualitative benefits assessment is presented in Section III of the document. Responses from the SAP, the USDA and other interested parties have been analyzed and are addressed in Section IV of this document. Section V summarizes the Agency's decision concerning pesticide products containing benomyl and thiophanate-methyl. The regulatory position takes into account all comments from the SAP, the USDA and other interested parties. Because the new information did not necessitate significant changes to the regulatory positions in the Benomyl PD 2/3 and the Thiophanate-Methyl PD 2, the Agency determined that it was not necessary to have the SAP review the new studies.

The responses from the SAP and the USDA are presented in their entirety in Appendix A and Appendix B, respectively. All comments are available for review in the public file in the office of the Document Control Officer, Chemical Information Division, Room E 477, (TS-793), EPA, 401 M Street, S.W.; Washington, D.C.; 20460.

II. New Information and Revisions to the Risk Analyses

A. Oncogenicity Studies

1. Introduction

Data indicating existence of oncogenic effects were not available to the Agency when earlier position documents on benomyl and thiophanate-methyl were published (US EPA, 1977a, 1977b, 1979a, 1979b). Since then, the Agency has received one study on benomyl and two studies on MBC (a metabolite of both benomyl and thiophanate-methyl) indicating that these compounds have the potential to induce oncogenic effects in mice. These studies, their impact on the overall risks presented by benomyl and thiophanate-methyl, as well as their integration into the Agency's final determination regarding regulatory action on these fungicides are discussed in the following sections of this position document.

2. Benomyl Study

a. DuPont Chronic Feeding Study

On March 20, 1981, DuPont provided the Agency with preliminary information from their analysis of liver tissue samples collected during a two-year mouse feeding study with benomyl, which showed an increased incidence of hepatocellular neoplasms (Chen, 1981). On October 27, 1981, DuPont submitted the completed report on the review of all tissues from this study which concluded, "No new oncogenic effects were found during the complete histomorphologic evaluations" (Everett, 1981a). On January 27, 1982, DuPont submitted the final report of this study, which indicated that no tumorigenic effect was observed in any organ system other than the liver (Wiechman, 1982).

In this study, male and female CD⁻¹ mice were given diets containing 0, 500, 1,500 or 5,000-7,500 ppm benomyl. The concentration of benomyl in the diet of the high-dose group was reduced from 7,500 ppm to 5,000 ppm after 37 weeks on test because marked reductions in mean body weights and weight gains were observed in both male and female mice. DuPont scientists concluded, "Benomyl administration in the diets of male and female mice under the conditions of this study resulted in a hepatic carcinogenic effect" (Weichman, 1982). Survival and the incidence of and time to first observation of palpable tissue masses among mice were comparable in test and control groups. Decreased body weight gains in male and female mice at the 1,500 ppm and 5,000-7,500 ppm groups when compared to controls as well as hepatotoxic changes related to benomyl treatment in the 5,000-7,500 ppm groups were reported. No clinical observations or hematological changes attributed to benomyl were reported.

Although alveolar cell carcinomas occurred at significantly increased rates in the low and intermediate dosed males ($p < 0.05$, Fisher's Exact Test), DuPont

scientists concluded that this increase was not an effect of compound administration based on the following information:

- (1) while statistically significant when compared to the pulmonary tumors in the benomyl control male mice (16%), the incidences of pulmonary tumors in the low and intermediate doses (30% and 29%, respectively), were within the range for incidence of such tumors in historical control animals from other such tests in their laboratory (20% to 36% in four other studies);
- (2) a dose response in the X^2 Test for Trend was not observed for pulmonary tumors in male mice receiving benomyl;
- (3) calculation of the median time-to-pulmonary tumor discovery in male mice did not provide evidence for a dose-related response for tumor latency;
- (4) the percent of total pulmonary tumors discovered in animals dying prior to terminal sacrifice did not demonstrate a dose response; and
- (5) a decrease in incidences of pulmonary tumors occurred in male mice treated with MBC compared to their concurrent control group (the metabolic and chemical behavior of benomyl and MBC are reported to be analogous by DuPont) (Everett, 1982).

The Agency has reviewed this study (Kasza, 1981a) and agrees with DuPont's pathologist that oncogenesis was established in the livers of male mice at low and intermediate dose levels and in female mouse livers at all dose levels. There was an increased incidence, not only in benign, but also in malignant hepatocellular and alveolar cell neoplasms in the male. A proportionally higher incidence in decrease of latency (the time interval between treatment and tumor appearance), both in female liver neoplasms and male lung neoplasms occurred. The presence of malignant liver tumors in males and females, the increased incidence in lung tumors in male mice, the decrease in latency in test animals, and the earlier occurrence of female liver tumors in test animals, compared with controls are supporting data for oncogenicity.

Regarding DuPont's conclusion on the incidence of pulmonary tumors in male mice, the Agency considered that it would be just as logical, when referring to the spontaneous incidence of pulmonary tumors in DuPont's historical controls, to remove the high value of 36% (Velpar® test) as an outlier as it would be to remove benomyl. The remaining four studies (Terbacil, MBC, DPX-4189, benomyl) would then have a mean for spontaneous pulmonary tumor incidence in controls of 20% with DPX-4198 being 4% higher and benomyl 4% lower than the mean (Litt, 1982a). As a consequence of its review of the DuPont study, the Agency noted that data exist to support the hypothesis of decreased latency for lung carcinoma in male mice only (Litt, 1982b).

3. MBC Studies

a. DuPont Chronic Feeding Study

On March 20, 1981, DuPont provided the Agency with preliminary information from their analysis of liver tissue samples collected during a two year mouse feeding study with MBC which showed compound related neoplastic effects in the livers of male and female mice (Everett, 1981b). On October 27, 1981, DuPont submitted the completed report on the review of all tissues from this study which concluded that "no new oncogenic effects were found during the complete histomorphologic evaluations" (Everett, 1981a). On January 27, 1982, DuPont submitted their final report of this study which indicated that no carcinogenic effect was observed in any organ system other than the liver (Wood, 1982).

In this study, male and female CD⁻¹ mice were given diets containing 0, 500, 1,500 or 7,500 ppm MBC. The concentration of MBC in the diet of the high dose male group was reduced from 7,500 to 3,750 during test week 66 and finally, the group was terminated during week 73. DuPont scientists concluded that "MBC administration in the diets of male and female mice under conditions of this study, resulted in a hepatic carcinogenic effect" (Wood, 1982). Male mice in the high dose group tended to consume more diet and had a lower food efficiency than male mice in the control group. Survival of male mice in the intermediate (1,500 ppm) and high (7,500-3,750 ppm) dose groups was significantly lower than that of male control mice. Male mice in the intermediate (1,500 ppm) and high (7,500-3,750 ppm) dose groups and female mice in the high (7,500 ppm) dose group exhibited compound-related histomorphological changes in the kidney. Degenerative or toxic changes were observed in the livers of male mice in the test groups. No effects on body weight, weight gain, clinical observations or hematological parameters that could be attributed to dietary administration of MBC were noted.

The Agency has reviewed this study and is in agreement with DuPont's pathologist that compound-related hepatic carcinogenicity was established in the male intermediate dose group (1,500 ppm). However, the Agency does not agree that a valid conclusion can be made regarding the high dose male group (7 500-3,750 ppm) since it was terminated before the majority of the tumors developed in other groups. In females, the hepatic oncogenic effect was compound and dose related at all dose levels. There was an increased incidence of both benign and malignant tumors in both sexes. Dose-related decreases in latency of tumor appearance were present in both sexes. The oncogenic effect of MBC was comparable to that of the parent chemical, benomyl (in both males and females, the incidence of hepatocellular neoplasms increased in test groups; there was no increased incidence in lung tumors with MBC) (Kasza, 1982b).

b. Beems, Til and van der Heijden

The Agency has reviewed an additional report on the oncogenicity of MBC (Beems, Til and van der Heijden, 1976) that is consistent with the results of DuPont's study on MBC. In this study, SPF, albino, Swiss random bred mice were fed diets containing 0, 150, 300 and 1,000 ppm MBC (high dose increased first to

2,000 ppm and then to 5,000 ppm at weeks 4 and 8, respectively) for 80 weeks. It was concluded that feeding MBC to mice in this study resulted in an increased incidence of malignant liver tumors in males and an increased occurrence of benign liver tumors in females at 5,000 ppm (Sochard, 1981a).

4. Thiophanate-Methyl Studies

No carcinogenic effects have been reported in existing chronic feeding studies with thiophanate-methyl. The highest dose used in these tests was 640 ppm in mice (Kosaka and Tsubura, 1973), 640 ppm in rats (US EPA, 1973) and 250 mg/kg/day in dogs (US EPA, 1973). However, these results cannot be compared to those with tests on benomyl or MBC since the levels tested with thiophanate-methyl are much lower than the doses for those two.

B. Mutagenicity Studies

1. Benomyl

a. Summary of PD 1 Information

In PD 1, the Agency presumed against benomyl registrations based upon evidence that it and the metabolite, MBC, caused mutagenic effects (US EPA, 1977a). PD 1 indicated that both benomyl and MBC caused chromosomal effects in mammalian cell cultures (Styles and Garner, 1974; Debrander et al., 1976) and mammalian tests in vivo (Styles and Garner, 1974; Seiler, 1976). In addition, benomyl caused chromosomal effects in plants (both higher and lower plants) (Zutshi and Kaul, 1975; Boyle, 1973; Dassenoy and Meyer, 1973; Richmond and Phillips, 1975; Davidse, 1973; Hammerschlag and Sisler, 1973).

PD 1 also indicated that benomyl caused point mutations in Fusarium oxysporum (Dassenoy and Meyer, 1973), that Benomyl and MBC caused point mutations in Salmonella typhimurium strains G46, TA1530 and TA1535 (without metabolic activation), and Escherichia coli strain WP2 uvrA, and the MBC caused mutations in Salmonella strain LT-2 (Seiler, 1972; Kappas et al., 1976). PD 1 also included a statement from Seiler (1973, 1975) indicating that point mutations appeared to result from incorporation of benzimidazoles into DNA in place of purine bases. One negative point mutation study was reported in PD 1 (Ficsor and Bordas, unpublished).

b. Summary of PD 2/3 Information

In PD 2/3, the Agency responded to rebuttals regarding mutagenicity. The Agency accepted DuPont's rebuttal of Styles and Garner (1974) which stated that insufficient data were presented in the study to support the claim of chromosomal breakage or anaphase bridges. In addition, DuPont cited several other studies as evidence that benomyl or MBC do not cause chromosomal effects (Dassenoy and Meyer, 1973; Sherman, Culik and Jackson, 1975; Hoffman and Peh, 1974; Mollet, 1976; Siebert, Zimmerman and Lemperle, 1970; Seiler, 1977). The Agency agreed that these studies supported the hypothesis that benomyl does not produce direct chromosomal damage. However, the Agency did not accept them as evidence that chromosomal effects could not result from other mechanisms such as spindle interference or mitotic interruption.

In regard to point mutations, the Agency accepted the DuPont rebuttal argument that Dassenoy and Meyer (1973) did not unequivocally demonstrate such activity. In addition, the Agency accepted DuPont's argument that Seiler (1972) contained certain omissions that limited its utility for determining the potential for point mutagenic activity and that Kappas et al. (1976) alone was inadequate to determine if benomyl was a point mutagen. Further, the Agency agreed that the studies by Seiler (1972, 1975) did not unequivocally demonstrate that benzimidazole is incorporated into DNA. Data from a mouse spot test (Fahrig and Seiler, 1979) were not definitive enough to demonstrate point mutagenicity in vivo.

DuPont submitted several studies as evidence that benomyl and MBC do not cause point mutations (Haskell Laboratory, 1977). The Agency concluded that the positive results in some of these studies could have been due to a contaminant. Further, the Agency discussed negative results in studies with Bacillus subtilis, Salmonella typhimurium, and Escherichia coli (Shirasu, Mariya and Kato, 1978) and a negative sex-linked recessive lethal test in Drosophila (Lamb and Lilly 1973).

The Agency concluded in PD 2/3 that the available evidence was insufficient to conclude that either benomyl or MBC react directly with DNA to cause point mutations or direct chromosomal aberrations in mammalian systems. However, the Agency indicated that available evidence supported the presumption that benomyl or its metabolite, MBC, were spindle poisons capable of inducing non-disjunction, and that benomyl and its metabolites may reach the mammalian gonad. This evidence provided an adequate basis for a qualitative presumption against benomyl for potentially heritable spindle effects. However, the Agency indicated that evidence was lacking to demonstrate definitively that benomyl induced such effects in germ cells, although preliminary information (Tates, 1978) indicated that MBC might cause non-disjunction in germ cells. The Agency indicated that persons exposed to high levels of benomyl might be at risk from induction of spindle effects. However, a means to quantify such risk was not available and the existing data were not adequate to demonstrate the existence of a significant risk at estimated exposure levels (US EPA, 1979a).

c. Data Review

Additional data bearing on the mutagenicity of benomyl were received subsequent to publication of the PD 2/3. These are summarized in Table 1. The Agency's conclusions regarding potential mutagenic risk presented by benomyl are discussed in Section II.F.2 of this document. Existing data on mutagenicity are summarized in this section.

1) Gene Mutations

Two studies provided evidence of no mutagenic activity in S. typhimurium (Ficsor et al., 1978; Shirasu et al., 1978), however, the dosage levels used were below the 5,000 to 10,000 ug/plate range at which activity was noted in the other studies. Similar results in S. typhimurium were reported for technical and analytical grade MBC. Mutagenic activity was reported at a dose range from 4,000 to 10,000 ug/plate for MBC.

Table 1

Results of Mutagenicity Assays of Benzo(a)Pyrone
Submitted Since Publication of Position Document 2/3

Test Organism	Results/Comments	Reference
Mouse lymphoma LS178Y cells <u>in vitro</u>	Dosed by gavage at 250, 500, or 1,000 mg benzo(a)pyrene/kg on two consecutive days. Reported a statistically significant increase in number of micronuclei in bone marrow from femur bones at 48 hours in 250 and 500 mg/kg dose groups and 1,000 mg group at 24 and 48 hours.	Jotz et al., 1980
Chinese hamster ovary cells <u>in vitro</u>	Dose related increase in mutation frequency at TK locus which was enhanced by use of metabolic activation.	Evans and Mitchell, 1980
<u>Salmonella typhimurium</u>	Weakly positive results for sister chromatid exchange were reported.	Donovan and Krahn, 1981a
<u>S. typhimurium</u>	Benzo(a)pyrene was not mutagenic at doses as high as 250 ug per plate in strains TA1535, 1537, 98, and 100.	Horst and Krahn, 1980
<u>S. typhimurium</u>	Plates were treated with from 100 to 10,000 ug of benzo(a)pyrene. Number of revertants/plate increased by factor of 2.6 and 7.6 times in TA1537 and from 2 to 10 times in TA98.	Donovan and Krahn, 1981b
<u>S. typhimurium</u>	Plates were treated with from 100 to 10,000 ug benzo(a)pyrene. The number of revertants/plate increased from 4.2 to 8.95 times in the trials with positive responses in TA98 and from 3.7 to 6.4 times in TA1537 (all with activation).	Donovan and Krahn, 1981c
Chinese hamster ovary cells <u>in vitro</u>	MPC tested under procedures in same author citation at 1981b. Not mutagenic.	Fitzpatrick and Krahn, 1980
Chinese hamster ovary cells <u>in vitro</u>	Looked for mutations at the HGPRT locus. Benzo(a)pyrene was not mutagenic under these test conditions.	Waters and Krahn, 1980
R6C3F1 Mice and F344 Rats	Looked for mutations at the HGPRT locus. No statistically significant differences in mutation frequency were noted with or without metabolic activation.	Tong, 1981a and 1981b

In regard to point mutations, the Agency accepted the DuPont rebuttal argument that Dassenoy and Meyer (1973) did not unequivocally demonstrate such activity. In addition, the Agency accepted DuPont's argument that Seiler (1972) contained certain omissions that limited its utility for determining the potential for point mutagenic activity and that Kappas et al. (1976) alone was inadequate to determine if benomyl was a point mutagen. Further, the Agency agreed that the studies by Seiler (1972, 1975) did not unequivocally demonstrate that benzimidazole is incorporated into DNA. Data from a mouse spot test (Fahrig and Seiler, 1979) were not definitive enough to demonstrate point mutagenicity in vivo.

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Table 1
Results of Mutagenicity Assays of Benomyl/MPC
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Test Organism	Results/Comments	Reference
Mice	Dosed by gavage at 250, 500, or 1,000 mg benomyl/kg on two consecutive days. Reported a statistically significant increase in number of micronuclei in bone marrow from femur bones at 48 hours in 250 and 500 mg/kg dose groups and 1,000 mg group at 24 and 48 hours.	Kirkhart, 1980
Mouse lymphoma L5178Y cells <u>in vitro</u>	Dose related increase in mutation frequency at TK locus which was enhanced by use of metabolic activation.	Jotz et al., 1980
Chinese hamster ovary cells <u>in vitro</u>	Weakly positive results for sister chromatid exchange were reported.	Evans and Mitchell, 1980
<u>Salmonella typhimurium</u>	Benomyl was not mutagenic at doses as high as 250 ug per plate in strains TA1535, 1537, 98, and 100.	Donovan and Krahn, 1981a
<u>S. typhimurium</u>	Plates were treated with from 100 to 10,000 ug of benomyl. Number of revertants/plate increased by factor of 2.6 and 7.6 times in TA1537 and from 2 to 10 times in TA98.	Horst and Krahn, 1980
<u>S. typhimurium</u>	Plates were treated with from 100 to 10,000 ug MPC. The number of revertants/plate increased from 4.2 to 8.95 times in the trials with positive responses in TA98 and from 3.7 to 6.4 times in TA1537 (all with activation).	Donovan and Krahn, 1981b
<u>S. typhimurium</u>	MPC tested under procedures in same author citation at 1981b. Not mutagenic.	Donovan and Krahn, 1981c
Chinese hamster ovary cells <u>in vitro</u>	Looked for mutations at the HGPRT locus. Benomyl was not mutagenic under these test conditions.	Fitzpatrick and Krahn, 1980
Chinese hamster ovary cells <u>in vitro</u>	Looked for mutations at the HGPRT locus. No statistically significant differences in mutation frequency were noted with or without metabolic activation.	Waters and Krahn, 1980
BC3F1 Mice and F344 Rats	Benomyl was tested for DNA repair using primary hepatocyte cultures. Benomyl did not induce DNA repair in either rat or mouse.	Tong, 1981a and 1981b

Table 1 Continued

Test Organism	Results/Comments	Reference
B6C3F1 Mice and F344 Rats	MBC was tested for DNA repair using primary hepatocyte cultures. MBC did not induce DNA repair in either rat or mouse.	Tong, 1981c and 1981d
<u>S. typhimurium</u> and <u>Streptomyces coelicolor</u>	Spot tests on plates with <u>Salmonella</u> strains TAI535, 1536, 1537, 1538 and <u>Streptomyces</u> . No mutagenic activity was reported for benomyl in either organism.	Carere et al., 1978
<u>S. typhimurium</u>	Series of tests: spot, liquid culture, and host mediated assays using strains <u>his</u> G46, TAI530, TAI535, TAI950 using both benomyl and MBC (11 samples from different sources; technical and formulated). No mutagenic activity was noted in any of these tests except one weak positive in <u>his</u> G46 with MBC was reported.	Fiscor et al., 1978
<u>S. typhimurium</u>	Bacterial assays with benomyl and MBC Strains TA98, TA100, TAI535, TAI537, and TAI538. Doubtful mutagenic activity was reported for both benomyl and MBC both with and without metabolic activation.	Eroegovich and Rashid, 1976 and Rashid and Eroegovich, 1977
<u>Saccaromyces cerevisiae</u>	Results of this test were reported for only one dose level. Positive results were reported but information presented in the study was insufficient to allow evaluation of the results.	Guerzoni et al., 1976
<u>Aspergillus nidulans</u> and <u>Cladysporium cucumerinum</u>	Mutagenic activity was reported in <u>A. nidulans</u> at pH 5.2 and 5.3 and in <u>C. cucumerinum</u> at pH 6.8. MBC was tested in this assay.	Nirenberg and Speakman, 1981, and Speakman and Nirenberg, 1981
<u>A. nidulans</u>	Benomyl tested in an excision repair deficient strain. A dose dependent effect was reported.	Kappas and Bridges, 1981
<u>S. cerevisiae</u> and <u>A. nidulans</u>	Mitotic gene conversion study was negative when benomyl with metabolic activation was tested.	de Bertoldi et al., 1980
<u>A. nidulans</u>	Negative for nondisjunction or crossing over. Increased frequency of segregants due to spindle inhibition reported.	de Bertoldi and Griselli, 1980
<u>Drosophila melanogaster</u>	Genetic toxicity tested in <u>Drosophila</u> . Noted sterility in some broods. This was considered to be consistent with spindle effects of both benomyl and MBC which were tested.	Lamb and Lilly, 1980

Table 1 continued

Test Organism	Results/Comments	Reference
Human lymphocyte cell culture	Grown in culture medium containing 0.5 mg MMC. No compound related chromosome aberrations were noted.	Lamb and Lilly, 1980
Human leukocyte cell cultures	Primary cultures were treated with benomyl. Cells examined in the high dose cultures did not show a statistically significantly increased incidence of chromosomal aberrations.	Gupta and Legator, 1975
Rats	Bone marrow and cultures of embryo cells of pregnant rats were examined for chromosomal aberrations after treatment with benomyl on days 7 to 14 of gestation. No increases in frequency of cells with chromosomal aberrations were reported in bone marrow cells. Embryonic cells had 5 times the chromosomal damage at the two highest doses (200, 500 mg/kg) than controls.	Ruzicka et al., 1976
Human	Peripheral blood cells from workers at a benomyl (Fundazole 50WP) plant were examined. No effects attributable to benomyl reported.	Ruzicka et al., 1976

Several reports on the mutagenic activity of benomyl and MBC in fungi (Dassenoy and Meyer, 1973; Guerzoni et al., 1976; and Kappas and Bridges, 1981) suggest that benomyl can induce gene mutations in Aspergillus nidulans and such activity has also been reported in A. nidulans and C. cucumerinum when MBC was tested (Nirenberg and Speakman, 1981; and Speakman and Nirenberg, 1981). However, purity of the benomyl and MBC which was tested was not established, therefore, a comparison of these results cannot be made with those results obtained in bacterial tests. These results also need to be considered in light of the studies by Kumari et al. (1977) which suggest that benomyl and MBC do not directly interact with DNA in fungi. (See discussion under primary DNA damage).

In vitro mammalian cell assays present uncertain results for benomyl in that no genetic effects were noted in Chinese hamster ovary cells (Fitzpatrick and Krahn, 1980) while the fungicide was mutagenic in mouse lymphoma cells (Jotz et al., 1980). One study with MBC in Chinese hamster ovary cells has been reported but no gene mutation induction was noted (Waters and Krahn, 1980).

No sex-linked recessive lethal mutations were reported by Mollet (1976) or Lamb and Lilly (1980) in Drosophila melanogaster exposed to benomyl or MBC. However, these studies need confirmation from other test systems or repeated assay with Drosophila.

2) Primary DNA Damage

No effect was observed in a Bacillus subtilis differential toxicity assay reported by Shirasu et al. (1978) or a mitotic gene conversion study in Saccharomyces cerevisiae (Siebert et al., 1978), however, dosages tested in these studies were not at or near cytotoxic levels established in other test systems. However, a study by de Bertoldi et al. (1980) showed that benomyl did not induce mitotic gene conversions in S. cerevisiae or A. nidulans.

Studies of the uptake of radiolabelled phosphorous into DNA, DNA melting profiles, and occurrence of ¹⁴C-label in DNA after exposure of fungi to labelled MBC suggested that the fungicide and its metabolite do not directly interact with DNA (Kumari et al., 1977). The study indicated that interference with the processes of DNA precursor biosynthesis without becoming part of the macromolecule itself occurred. These findings suggested that mutagenic activity of the fungicide and its metabolite noted in fungi is likely to result from a contaminant in the test material.

An increased frequency of sister chromatid exchanges in Chinese hamster ovary cells in vitro was reported by Evans and Mitchell (1980) after exposure of the cells to benomyl with and without metabolic activation. However, the response was described as weak.

In vitro studies were conducted with benomyl and MBC to determine the effect of the chemicals on DNA synthesis in primary rat and mouse hepatocyte cultures (Tong, 1981a, b, c, and d). Neither chemical induced DNA synthesis.

3) Chromosome Damage

The Agency originally cited reports in which benomyl and MBC were shown to damage chromosomes. Rebuttal arguments were accepted by the Agency against some of the studies, but additional data in support of the presumption have been found. Richmond and Phillips (1975) noted the occurrence of chromosomal aberrations (bridges). Zutshi and Kaul (1975) reported observing metaphase aberrations in barley exposed to Benlate and isolated breaks and chromatid exchanges in treated broad beans have also been observed.

Cultured human leukocytes treated with benomyl had more chromosomal aberrations than untreated cultures (Gupta and Legator, 1975), while in a separate experiment, human leukocytes did not have increased frequencies of chromosomal aberrations when treated with MBC (Lamb and Lilly, 1980).

In vivo cytogenetics studies were reported in several animal systems. No effects on chromosomes of bone marrow cells were reported in rats treated with Fundazole 50 WP (Ruzicska et al., 1975). No effects were noted (Seiler, 1976) on chromosomes in bone marrow cells from Chinese hamsters and mice treated with MBC.

Doses as high as 2500 ppm in the diet of rats did not induce dominant lethal effects (Sherman et al., 1975). Similar studies were conducted with MBC in mice (BASF, 1973; Hoechst, 1974; and BASF, 1975) (see PD 2 for thiophanate-methyl). These studies were negative.

A micronucleus test reported by Kirkhart (1980) showed that benomyl increased the number of micronuclei in polychromatic erythrocytes in treated mice. However, the effect could result from spindle effects (see Spindle Effects section below).

4) Chromosomal Nondisjunction

Chromosomal nondisjunction was observed in A. nidulans exposed to Benlate® 50 WP and benomyl (Dassenoy and Meyer, 1973; Kappas et al., 1974). MBC was also found to induce chromosomal nondisjunction in A. nidulans.

Benlate, benomyl, and MBC were reported to induce lagging chromosomes at telophase in mitosis of onion root tip cells. Boyle (1973) reported that the metaphase chromosomes were more condensed than normal.

5) Spindle Effects

MBC has been found to interfere with polymerization of microtubulin which is needed for the formation of spindle fibers in cell division. Many of the results from tests evaluating chromosomal effects may arise from affected cell division processes. Therefore, those studies could be reflective of microtubule inactivation. Chromosomal nondisjunction, lagging chromosomes, multinucleation, micronuclei formation, and chromosomal breaks and aberrations could result from microtubule inhibition during cell division.

2. Thiophanate-Methyl

Studies in plants (Noguchi et al., 1974; Buchenauer et al., 1973), animals (Palmer et al., 1972) and soil (Fleeker et al., 1974) have shown that thiophanate-methyl is metabolized to MBC. In its PD 1 on thiophanate-methyl (US EPA, 1977b), the Agency cited studies with MBC by Debrabander et al. (1976) and by Styles and Garner (1974) in mammalian cell cultures, and studies by Styles and Garner (1974) and Seiler (1976) in mammalian in vivo systems, as evidence of thiophanate-methyl's potential to cause chromosomal effects. The Agency also cited negative dominant lethal work by Makita et al. (1973) in mice, as well as studies that indicate that MBC interfered with nuclear division in fungi (Seiler, 1976; Makita et al., 1973; Hammerschlag and Sisler, 1973) in its PD 1. The Agency also indicated in PD 1 that MBC had the potential to cause point mutations (Seiler, 1972; Kappas et al., 1976; Seiler, 1973 and 1975).

On the basis of the above studies the Agency concluded in PD 1 that the thiophanate-methyl metabolite, MBC, was mutagenic and that a Rebuttable Presumption Against Registration of products containing that active ingredient had arisen.

In its PD 2 (US EPA, 1979b) the Agency, after an evaluation of rebuttals to the PD 1, concluded that existing evidence was insufficient to demonstrate that MBC reacts directly with DNA to cause point mutations or chromosomal aberrations in mammalian systems. However, the Agency concluded that MBC is a spindle poison capable of inducing non-disjunction which could result in aneuploidy or polyploidy, both chromosomal aberrations of concern. (See the discussion of the Agency's rebuttal opinions in the benomyl section above for conclusions on the studies cited in this section).

As with benomyl, the Agency concluded that a qualitative presumption of risk existed against thiophanate-methyl's MBC metabolite-caused spindle effects. However, definitive evidence of such effects in germ cells was lacking. Further, existing data were not adequate to demonstrate the existence of significant risk at estimated exposure levels. Therefore, the presumption against thiophanate-methyl registrations was rebutted.

C. Teratogenicity Studies

The Agency considered three studies in concluding that the risk criterion for teratogenicity had been exceeded in its benomyl PD 1 (US EPA, 1977a). Schtenberg and Torchinski (1972) reported effects including brain hernias, hydrocephalia, microphthalmia and anophthalmia when benomyl was administered by gavage to Wistar rats (either on days 1 to 20 or days 7 to 15 of gestation). Doses of 125 mg/kg/day were teratogenic. The NOEL was 62.5 mg/kg/day. Torchinski (1973) also induced teratogenic effects in Wistar rats given a 145 mg/kg dose of benomyl by gavage on day 12 of gestation. PD 1 also discussed a study (Sherman, Culik and Jackson, 1975) which reported that a diet containing about 400 mg/kg/day benomyl was not teratogenic to Charles River-CD rats.

In PD 2/3 the Agency concluded that the risk criterion for teratogenicity had not been rebutted (US EPA, 1979a). The Agency considered the teratology studies discussed in PD 1 and additional studies received after its publication. These included Agency-sponsored studies showing teratogenic effects down to the lowest dose tested (gavage) 62.5 mg/kg/day (Short et al., 1979; Kavlock, 1979), a DuPont study showing no teratogenic effects in CHR-CD rats following dietary dosing of the benomyl metabolite MBC up to 500 mg/kg/day (Haskell Laboratory, 1978), and a study showing teratogenic effects in Sprague-Dawley rats following gavage administration of MBC (DelaTour and Richard, 1976). None of these studies established a firm NOEL for benomyl's induction of teratogenic effects. Therefore, both EPA and DuPont committed themselves to perform additional teratology studies on benomyl. The Agency assumed in PD 2/3 that the NOEL for teratogenic effects would not be less than the NOEL for spermatogenic effects (the most sensitive effect for benomyl). Therefore, for its risk estimate in PD 2/3 the Agency used the level of 7.5 mg/kg/day (from a 1969 test by Hornberger showing spermatogenic effects in rats following inhalation exposure to benomyl). The margins of safety (MOS) for exposed populations was then estimated to range from 21 to 380.

Since publication of the PD 2/3 the Agency has received additional studies on the teratogenicity of benomyl. Kavlock, Chernoff, and Gray (1980) showed teratogenic effects in Wistar rats following gavage dosing at 0, 15.6, 31.2, 62.5, and 125 mg/kg/day (days 7-16 of gestation). The lowest effect level (LEL) was 62.5 mg/kg/day and the NOEL was 31.2 mg/kg/day. A dietary dosing subpart of this study did not show teratogenic effects when rats were fed diets containing up to 500 mg/kg/day of benomyl. A study by DuPont (Staples, 1980) showed teratogenic effects when CHR-CD rats were given benomyl by gavage. Dose levels were 0, 3.0, 10.0, 30.0, 62.5, and 125.0 mg/kg/day/. The LEL was 62.5 mg/kg/day and the NOEL was 30 mg/kg/day. Unilateral microphthalmia was reported at 10 mg/kg/day in two animals in this study. Although the effect was biologically significant, its occurrence was not statistically significant at that level. This effect occurred at a higher dose level at a statistically significantly increased incidence. DuPont is currently performing a follow-up study (using doses of 30 mg/kg/day and below) to further examine occurrence of this eye effect following gavage dosing. The Agency has, therefore, provisionally set a NOEL of 30 mg/kg/day for use in its teratogenic risk assessment in section II.F.3. of this document. This risk will be reassessed if DuPont's follow-up study shows a need.

In its PD 1 and PD 2 for thiophanate-methyl, the Agency discussed a teratogenicity test in which this compound was given by gavage to ICR mice from days 1 to 15 of gestation (Makita et al., 1973). The highest dose tested was 1,000 mg/kg/day. The only observed malformation was cleft palate which occurred with the same incidence in control and experimental animals. This study was considered inconclusive since other organ systems were not examined for teratogenic effects.

D. Spermatogenic Effects Studies

In PD 1 the Agency considered a number of studies which identified the testes as a primary target of benomyl. In an acute toxicity test, Sherman and Krauss (1966) reported the degeneration of germinal tissue and aspermatogenesis in

rats receiving gavage treatment of benomyl. The lowest effect level (LEL) was 3400 mg/kg, the lowest dose tested. Sherman and Krauss (1966) also reported a reduction of sperm in mature rats receiving ten doses of benomyl at 200 mg/kg/day. Sherman (1965) also reported reduction of sperm in rats receiving a single 670 mg/kg benomyl dose by gavage.

Sperm production was not affected in chronic feeding studies in dogs (Sherman et al., 1970) and rats (Sherman et al., 1969) receiving benomyl at doses of up to 2500 ppm in the diet. Further, the fertility index was not affected in a three-generation reproductive effects study in rats receiving up to 2500 ppm benomyl in the diet (Sherman et al., 1975).

PD 1 also reported on the results of inhalation studies with benomyl on rats (Hornberger, 1969) and dogs (Littlefield, 1969) which showed a reduction of spermatogenic activity. The lowest NOEL (7.5 mg/kg/day) occurred in the rat study (Hornberger, 1969). In PD 2/3 (US EPA, 1979a) the Agency concluded that the risk criterion had not been rebutted. The margins of safety (MOS) for this effect were based upon the NOEL of 7.5 mg/kg/day via the inhalation route of exposure (Hornberger, 1969). These ranged from 21 to 380.

In a study by Carter and Laskey (1982) adult Sprague-Dawley rats were given ten daily doses of 0, 200 or 400 mg benomyl/kg/day by gavage. Various parameters were measured 14 days after treatment ended. Carter and Laskey reported statistically significant depression in the total epididymal (caudal and caput) sperm counts and in the vas deferens sperm concentration in rats treated with 200 to 400 mg/kg/day. A slight to severe generalized hypospermatogenesis in two animals and a slight to moderately severe hypospermatocytogenesis in test animals was reported in the six animals given histological evaluation of the testes (dose level was 400 mg/kg/day). In addition, caudal epididymis weights were significantly depressed with benomyl treatment.

However, Carter, Hein and Laskey (1982) reported that ten daily gavage treatments of prepubertal Sprague-Dawley rats with 0 or 200 mg benomyl/kg/day did not cause damage to the reproductive system while the same exposure in adults caused significant reproductive effects (Carter and Laskey, 1982).

Kavlock et al. (1982) reported a decrease in testicular weights at 31.2 mg/kg/day in the offspring of Wistar rats receiving gavage treatments of benomyl during both gestation and lactation. No effects on any parameters were evident in rats receiving 15.6 mg/kg/day by gavage. The above studies all show effects at levels higher than those reported by Hornberger (1969). Therefore, the NOEL from that study is used in the spermatogenic effects risk assessment in this document (see Section II.F.4).

When thiophanate-methyl was tested for effects on the male reproductive system, negative effects were observed at doses as high as 195 mg/kg/day. Little change occurred in the weights of the accessory sex organs, though there were significant increases in the weight of the prostate gland (Thomas and Schein, 1974). Decreased spermatogenesis was reported in a thiophanate-methyl chronic feeding study at 640 ppm. The NOEL for this effect was 160 ppm (Anonymous, undated).

E. Revised Exposure Estimates

1. Background

In addition to the new toxicology data, other information has come to the Agency's attention since 1979 which necessitates changes in or amendments to the exposure estimates presented in the PD 2/3 on benomyl and the PD 2 on thiophanate-methyl.

The Agency indicated in PD 1 (US EPA, 1977a) that benomyl hydrolyzed to MBC in aqueous solution under laboratory conditions (Anonymous, 1974). Further study indicated that in aqueous solution benomyl rapidly breaks down to MBC with conversion being complete within one week (Peterson and Edington, 1969). However, benomyl is relatively stable in spray suspension. Benzimidazole-containing residues of benomyl can be found in soil although most parent benomyl rapidly disappears. Their half-life is three to six months on turf and six to twelve months on bare soil with the major portion of the metabolites being found in the top four inches of the soil (Baude et al., 1974). Benomyl and its metabolites do not leach or move significantly from the site of application (Rhodes and Long, 1974). Benomyl remains on foliar surfaces as a relatively stable residue (Baude et al., 1973). Relatively little benomyl penetrates leaves. That which does is translocated as MBC and 2-aminobenzimidazole (2-AB) (Hammerschlag and Sissler, 1972 and 1973). MBC is taken up into plants when benomyl is applied to the root area. Only a small amount of benomyl was found in the flesh of oranges stored two months after benomyl dipping. The major portion remained in the peel (Lowen, 1974).

Benomyl is normally metabolized in animals to 5-hydroxy-2-benzimidazolecarbamate (5-HBC), 99 percent of which appears in the urine and feces of dogs or rats within 72 hours of administration of a single oral dose. Other possible metabolites include MBC and 4-hydroxy-2-benzimidazolecarbamate (4-HBC). Benomyl and its metabolites do not accumulate in animal tissues but are eliminated rather rapidly as sulphate or glucuronide conjugates in the urine or feces (Gardiner et al., 1974; Douch, 1973). The Agency indicated in PD 2/3 (U.S. E.P.A., 1979a) that benomyl or its metabolites can reach the mammalian gonads (Styles and Garner, 1974; Gardiner et al., 1974).

The Agency indicated in PD 1 (US EPA, 1977b) that thiophanate-methyl was stable in acidic solution and unstable in alkaline solution. Thiophanate-methyl and its metabolites are essentially immobile in the soil (US EPA, 1977d). Thiophanate-methyl converted completely to MBC in 0.066 M phosphate buffer, pH7, at 70°C in 26 hours (Vonk and Sijpesteijn, 1971). In moist soil thiophanate-methyl was degraded rapidly; 80 to 90 per cent had disappeared from soils five days after application. The principle soil metabolite was MBC (Flecker et al., 1974). Thiophanate-methyl was converted to MBC after uptake through roots and after foliar treatment of bean seedlings (Noguchi et al., 1974).

The metabolism of TM in animals is qualitatively similar to animal metabolism of benomyl. Feeding of radiolabeled thiophanate methyl to rats, mice, and dogs gave 80 to 100 per cent recovery of administered radioactivity in the feces and

urine within 96 hours (Noguchi et al., 1974; Fujino, et al., 1974). Radiolabeled thiophanate methyl was administered at 45 ppm in the diet of rats for 20 consecutive days (Kosaka, et al., 1975). An average of 90 per cent of administered radioactivity was excreted into urine and feces every day. Forty per cent was 5-HBC and 5 per cent was MBC. In a single dose study, Douch (1974) reported that MBC was the major excretion product in mice (30 per cent) and sheep (26 per cent), with less 5-HBC reported (20 per cent in mice, 9 per cent in sheep). Finally, in a 10 day feeding study of a lactating cow, Kosaka, et al. (1976) reported that most of the labeled material was excreted in the urine and a very small amount was excreted in the milk (0.61 per cent equivalent to 0.10 ppm total residue in the milk). 5-HBC was 48 per cent of the milk residue, whereas MBC and unchanged TM were only about 10 per cent of the milk residue. Thus, metabolism of TM in animals is qualitatively similar to benomyl, with quantitative variations depending on species and/or dosing.

2. Non-Dietary Exposure

a. Benomyl

In PD 1 (US EPA, 1977a) the Agency calculated benomyl exposure to applicators (inhalation only) from limited data to be 0.24 mg/kg for four hours of spraying. Home-use of benomyl was estimated to give an exposure of 0.48 mg/kg per application for a 60 kg human. Dermal penetration of benomyl was assumed to be 10 per cent.

In PD 2/3 estimates were revised by the Agency based upon comments received in response to the PD 1 and data submitted by DuPont. Even so, most of the PD 2/3 exposure estimates were still based upon data from studies with other chemicals. The exception to this was the estimate for exposure during home-use. In addition, DuPont submitted data indicating that dermal absorption of benomyl is very limited (Belasco, 1979). In this study there was only an 18-fold increase in benomyl blood levels in response to a 1,000-fold dose increase. The concentration of benomyl in the blood was low because of slow absorption coupled with rapid metabolism and elimination.

Based upon this study the Agency calculated that no more than 6.23 ug/kg benomyl could be absorbed after eight hours mixing/loading for aerial application. This was based upon the worst case dermal estimate of 1.8 mg benomyl/kg/hour provided by Day (1978). A number of assumptions utilized in deriving the maximum absorption of 6.23 ug benomyl/kg/8 hour day make this overestimate the likely case. These include the assumption that the total quantity of benomyl impinging upon the skin would stay on the skin for the full eight hours, the assumption that human and rat skin are equally permeable to benomyl, and the assumption that the dose reflected in the rat study used in this extrapolation was applied at the beginning of each hour.

Therefore, the Agency concluded that the dermal absorption of benomyl was in and of itself an insignificant dose during spraying operations, and it did not need to be considered for purposes of exposure calculation (Zendzian, 1979).

Even so, a proportion of this amount was included in the estimate of mixer/loader exposure based on the hours that these people were assumed to be

exposed per day. For all other activities the amount absorbed was assumed to be mathematically insignificant and was discounted (US EPA, 1979a).

Based on the above, the total body dose was calculated from data reflecting the number of hours worked by persons using benomyl as follows (US EPA, 1979a, PD 2/3) (included in this estimate is an assumed background exposure to benomyl from the diet of 0.02 mg/kg/day): aerial application (mixer/loaders, 0.26-0.35 mg/kg/day; pilots, 0.02 mg/kg/day; flaggers, 0.02 mg/kg/day); air blast (commercial application, 0.02-0.03 mg/kg/day; private application, 0.02-0.03 mg/kg/day); ground application (commercial, 0.02 mg/kg/day; private, 0.02 mg/kg/day); and home use, 0.02 mg/kg/day (see Table 2). For most of the uses the work-related increment of total exposure is lost in rounding when it is added to the dietary background of 0.02 mg/kg/day.

DuPont submitted a study which evaluated the inhalation exposure during benomyl mixing and loading for aerial application (Holt et al., 1979--also published as Everhart and Holt, 1982). DuPont stated that exposure would average 0.016 mg/kg/mixing cycle with a maximum expected value of 0.046 mg/kg/mixing cycle. The average exposure in the surrogate data used as the basis for the Agency's estimate in PD 2/3 was 2.5 mg/hr (0.04 mg/kg/hr). The average based on this DuPont study is 1.2 mg/hr with ranges from 0.06 mg/hr to 4.6 mg/hr.

If exposure is calculated based upon DuPont's data showing an average of 1.2 mg/hr (Everhart and Holt, 1982) and an assumption that 500 pounds of benomyl are loaded and mixed per day (requiring about 34 minutes of actual mixing) then the average daily exposure would be about

$$\frac{(1.2 \text{ mg/hr}) \times (0.56 \text{ hr/day})}{60 \text{ kg/person}} = 0.01 \text{ mg/kg/day.}$$

If the estimate, extrapolated from DuPont's data, is based upon pounds loaded, average daily exposure would be about

$$\frac{\left[\frac{0.08 \text{ mg}}{42 \text{ lbs}} \right] \times (500 \text{ pounds/day})}{60 \text{ kg/person}} = 0.016 \text{ mg/kg/day.}$$

USDA estimated that as much as 1,000 acres of soybeans could be treated aerially with benomyl in one day. Using the DuPont data, this permits the calculation of an estimate ranging from 0.02 mg benomyl/kg/day (based on time spent loading) to 0.03 mg benomyl/kg/day (based on pounds loaded). This is about an order of magnitude lower than the existing estimate which was based upon an assumption of 6 hours involved in such work per day. Evidence therefore suggests that the original worst case estimates might be decreased by about an order of magnitude if more precise data on the quantity of benomyl actually loaded or the hours involved in actual loading per day were known.

b. Thiophanate-Methyl

The Agency estimated exposure to thiophanate-methyl in its PD 2 from data developed on a surrogate chemical which indicated that for air blast application dermal exposure would be 0.2 mg/kg/hr and for inhalation exposure

Table 2 Continued

Category	Hours Per Day	Total Exposure Without Dust Mask (mg/kg/day) ²
Ground Boom Application		
Turf*	6	0.02
Berries Private	3	0.02
Wheat	8	0.02
Home Use	0.05	0.02
Mushrooms	4.5	0.02
Turf-Handspray	0.5	0.02
Vegetables*	2	0.02
Reentry		
Adult (60 kg)	7.5	0.02
Minor (40 kg)	7.5	0.02

*Thiophanate-methyl registered use. It is assumed that worker exposure to thiophante-methyl would be no greater than that estimated for benomyl.

1/

Data submitted by DuPont suggest that exposure estimates for mixer/loaders in this table might be an order of magnitude high. Precise data on the amount of fungicide mixed and the actual time involved therein, would permit the Agency to confirm this.

2/

As an example, these estimates are calculated as follows for mixer/loaders for aerial application in grapes.

$$\begin{aligned}\text{Inhalation exposure} &= (0.04 \text{ mg/kg/hr}) \times (8 \text{ hours}) \\ &= 0.32 \text{ mg/kg/day}\end{aligned}$$

$$\begin{aligned}\text{Total exposure} &= (\text{Inhalation exposure}) + (0.02 \text{ mg/kg/day dietary background}) + \\ &\quad (0.006 \text{ mg/kg/day dermal penetration}) \\ &= 0.346 \text{ or } 0.35 \text{ mg/kg/day}\end{aligned}$$

Exposure from postharvest use on citrus, use on pineapples and sugarcane cause only negligible exposure due to apparatus associated with use (US EPA, 1979a; Sochard, 1981).

Table 2

Estimated Work-Related Exposure for Benomyl and Thiophanate-Methyl¹

Category	Hours Per Day	Total Exposure Without Dust Mask (mg/kg/day) ²
Aerial Application		
Grapes/Fruit Crops*		
Mixer/loaders	8	0.35
Pilots	3	0.02
Flaggers	8	0.02
Wheat		
Mixer/loaders	7	0.31
Pilots	3	0.02
Flaggers	7	0.02
Rice/Soybeans*/ Stone Fruits*		
Mixer/loaders	6	0.26
Pilots	3	0.02
Flaggers	6	0.02
Berries		
Mixer/loaders	5	0.22
Pilots	3	0.02
Air Blast Application		
Stone Fruits*		
Commercial	7	0.03
Private	8	0.03
Grapes		
Commercial	3	0.02
Private	4	0.02
Fruit Crops*		
Private	6	0.02
Berries		
Commercial	3	0.02
Citrus	8	0.03

0.005 mg/kg/hr. In this estimate the Agency assumed 0.5% dermal penetration of thiophanate-methyl. Exposure was calculated as follows (does not include dietary exposure): ornamentals (commercial application, 0.00665 mg/kg/day; private application, 0.00665 mg/kg/day); turf (commercial application, 0.135 mg/kg/day; private application, 0.038 mg/kg/day), and stone fruits (commercial application, 0.21 mg/kg/day; private application, 0.21 mg/kg/day) (US EPA, 1979b).

3. Dietary Exposure

a. Benomyl

In Position Document 1, the Agency assumed that the average person would consume 0.03 mg/kg of benomyl or its benzimidazole metabolites per day. This amount represented the maximum dietary exposure that would result when residues of benomyl were present in individual foods at tolerance levels and when all foods were assumed to be treated.

In PD 2/3 the Agency amended this dietary exposure estimate to reflect additional data. That document indicated that a more realistic estimate of dietary exposure to a 60 kg person eating 1.5 kg of food daily would be 0.02 mg/kg/day of benomyl or its benzimidazole metabolites (Johnson, 1979). MBC may also be present on crops which have been treated with thiophanate-methyl, however, 40 CFR 180.3(d)(10) prohibits the benzimidazole moiety from exceeding the highest established tolerance for a pesticide having this metabolite.

The Agency has modified the existing dietary exposure estimate to reflect additional information. The revised dietary exposure analysis (Table 3) indicates a worst case dietary exposure of about 2.0 mg benomyl or MBC/day/person (0.03 mg/kg/day for a 60 kg person). This assumes that all crops which receive benomyl treatments contain residues at tolerance level and that 100 percent of these crops are treated. This estimate considers established seed treatment uses (corn, onions, sweet potatoes, spinach, cabbage, cauliflower, broccoli, kale, collards, Brussels sprouts, turnips, rutabagas, mustard greens, Chinese cabbage, garlic, kohlrabi), and a number of tolerances that are pending establishment (wheat, oats, barley, rye, yams, currants, and papayas). When this figure is corrected for the percentage of crops with established benomyl tolerances that actually receive such treatments the estimate is about 0.9 mg/day/person (about 0.02 mg/kg/day for a 60 kg person) (Reinert, 1981).

DuPont studies demonstrated that residues of benomyl in the seed and transplant treatments would be less than the limit of sensitivity of the analytical methodology (0.2 ppm) and therefore would present a minimal health risk (US EPA, 1981).

The Agency has also evaluated DuPont's data on the average residues measured on a limited number of commodities receiving foliar and/or post-harvest treatments of benomyl. The Agency considered it appropriate to base the average residues on crops that received post-harvest and foliar treatments when available. This results in higher residues than foliar treatments alone, since most fruits receive post-harvest treatments. Dietary exposure based on such average residues is estimated at 0.2 mg/day/person (0.003 mg/kg/day based on a 60 kg person) (Saito, 1982c).

The preceding dietary estimates were used by the Agency in estimating the oncogenic risk to consumers. These estimates, in effect, represent exposure on the basis of average amounts of food ingested on an annual basis. Since teratogenic and reproductive effects can result from a single exposure on a given day, the Agency determined the maximum amount of benomyl that could be ingested in a normal single serving of the commodities having benomyl tolerances. These are presented in Table 4 and range from 0.00005 mg/kg body weight/serving to 0.118 mg/kg body weight/serving for a 60 kg person.

b. Thiophanate-Methyl

The Agency estimated dietary exposure to thiophanate-methyl and its benzimidazole metabolites to be about 0.005 mg/kg/day based on tolerances then established for strawberries and stone fruits. Since then the Agency has granted new uses for thiophanate-methyl that are basically the same as those for benomyl (with some exceptions). Therefore, it is reasonable to assume that dietary exposure to thiophanate-methyl and its MBC metabolite would now be similar and not greater than that from benomyl (thiophanate-methyl uses are shown in Tables 3 and 4).

F. Revised Risk Estimate

1. Oncogenic Risk

a. Derivation of the Potency Estimator

The Agency had no data indicating that benomyl was an oncogen prior to publication of PD 2/3. When the Agency received data which showed that benomyl and its metabolite, MBC, were hepatocarcinogenic in mice, it became necessary to calculate the oncogenic risk presented to humans by this compound.

In its assessment of benomyl's oncogenic risk, the Agency evaluated long term feeding studies in mice with benomyl (Wiechman, 1982) and MBC (Wood, 1982). The details of these studies are discussed in Section II.A. of this position document. The Agency's quantitative risk estimate was based upon data on hepatocellular adenomas and carcinomas in female mice fed 500 and 1500 ppm MBC, since these were found to be the most sensitive groups for this tumor type in either the Wood (1982) or the Wiechman (1982) studies.

When using mouse liver data as the basis for extrapolating cancer risks to man, it is important to consider all relevant ancillary data available. No positive cancer effects have been reported in benomyl tests with rats. In mice, liver tumors were reported in both sexes in one study with benomyl (Weichman, 1982) and two studies with its metabolite, MBC, (Wood, 1982; Beems, Til and van der Heijden, 1976). Evidence also exists to support the hypothesis of a decreased latency for lung carcinomas in male mice treated with benomyl (Weichman, 1982). Data also show that under selected conditions, benomyl metabolites have mutagenic potential (e.g., weakly positive effect in a sister-chromatid exchange study and mouse lymphoma cell point mutation). The liver has been reported to be a target organ for various non-oncogenic effects from benomyl in several species. Target sites for additional effects are the reproductive system and blood.

Table 3
Worst Case Dietary Exposure for Benomyl and Thiophanate-Methyl

Crop	Tolerance (ppm) ¹	Food Factor (%)	Portion of the Crop Treated with Benomyl (%)	Average Daily Ingestion of Benomyl (mg/day person) Worst Case	Corrected for the Percent Crop Treated
Pineapple	35.0	0.30	33.0	0.15560	0.05135
*Apricots	15.0	0.11	73.0	0.02529	0.01846
*Cherries	15.0	0.10	36.0	0.02299	0.00828
*Nectarines	15.0	0.03	74.0	0.00675	0.00500
*Peaches	15.0	0.90	74.0	0.20235	0.14974
*Plums, incl. prunes	15.0	0.13	17.0	0.02989	0.00508
Citrus Fruits	10.0	3.81	66.0	0.57179	0.37738
Grapes, not raisins	10.0	0.45	29.0	0.06745	0.01956
Raisins	50.0	0.04	29.0	0.03066	0.00889
Mushrooms	10.0	0.03	50.0	0.00450	0.00225
Blackberries	7.0	0.03	50.0	0.00315	0.00158
Blueberries	7.0	0.03	55.0	0.00315	0.00173
Boysenberries	7.0	0.03	50.0	0.00315	0.00158
Dewberries	7.0	0.03	50.0	0.00315	0.00158
Loganberries	7.0	0.03	50.0	0.00315	0.00158
Raspberries	7.0	0.03	27.0	0.00315	0.00158
*Apples	7.0	2.53	23.0	0.26565	0.00085
Pears	7.0	0.26	46.0	0.02683	0.06110
Rice	5.0	0.55	22.0	0.04139	0.01234
*Strawberries	5.0	0.18	70.0	0.01380	0.00911
Tomatoes	5.0	2.87	18.0	0.21561	0.00966
*Celery	3.0	0.29	88.0	0.01288	0.03700
Mangoes	3.0	0.03	50.0	0.00135	0.01133
*Beans	2.0	2.04	37.0	0.06120	0.00068
*Bananas	0.2	1.42	100.0	0.00426	0.02264
Avocados	1.0	0.03	5.0	0.00045	0.00002
Cucumbers,	1.0	0.73	44.0	0.01088	0.00479
incl. pickles					
Melons	1.0	2.00	80.0	0.03005	0.02404
Pumpkins, incl. squash	1.0	0.11	90.0	0.00169	0.00152
*Peanuts	0.2	0.36	22.0	0.00107	0.00024
*Soybeans	0.2	0.92	2.6	0.00275	0.00007
*Sugar, cane and beets	0.2	3.64	100.0	0.01091	0.01091
*Nuts	0.2	0.10	38.0	0.00031	0.00012
*Liver	0.2	0.03	100.0	0.00009	0.00009
*Eggs	0.1	2.77	100.0	0.00416	0.00416
*Milk and dairy	0.1	28.62	100.0	0.04292	0.04292
products	(1.0 t-m)				
*Meat, incl. poultry	0.1	13.85	100.0	0.02077	0.02077
Total				1.8971	0.93268
				(0.03160 mg/kg/day)	(0.0155 mg/kg/day)

Table 3 Continued

Crop	Tolerance (ppm) ¹	Food Factor (%)	Portion of the Crop Treated With Benomyl (%)	Average Daily Ingestion of Benomyl (mg/day person) Worst Case	Corrected for the Percent Crop Treated
Corn, sweet	0.2	1.43	100	0.00429	0.00429
Wheat ²	0.2	10.36	100	0.03109	0.03109
Oats ²	0.2	0.36	100	0.00107	0.00107
Barley ²	0.2	0.03	100	0.00009	0.00009
Rye ²	0.2	0.03	100	0.00009	0.00009
Onions ²	1.0	0.83	100	0.01242	0.00009
Sweet potatoes	0.2	0.40	100	0.00120	0.01242
Yams (yautia)	0.2	0.03	100	0.00009	0.00120
Spinach ²	0.2	0.05	100	0.00015	0.00009
Carrots ²	0.2	0.48	100	0.00144	0.00015
Curranis	7.0	0.03	100	0.00315	0.00144
Papayas ²	3.0	0.03	100	0.00135	0.00315
Cabbage, sauerkraut	0.2	0.74	100	0.00221	0.00135
Cauliflower	0.2	0.07	100	0.00021	0.00221
Broccoli	0.2	0.10	100	0.00031	0.00021
Kale	0.2	0.03	100	0.00009	0.00031
Collards	0.2	0.08	100	0.00025	0.00009
Brussel sprouts	0.2	0.03	100	0.00015	0.00025
Turnips	0.2	0.05	100	0.00009	0.00015
Rutabagas	0.2	0.03	100	0.00009	0.00009
Mustard greens	0.2	0.06	100	0.00018	0.00018
Chinese cabbage	0.2	0.03	100	0.00009	0.00009
Garlic	0.2	0.03	100	0.00009	0.00009
Eggplant	0.2	0.03	100	0.00009	0.00009
Peppers	0.2	0.12	100	0.00036	0.00009
Sum Total					0.00036
1.966 = mg/day/ person (0.0328 mg/kg/day)					1.00158 mg/day/ person (0.017 mg/kg/day)

¹ Tolerances also established for thiophanate-methyl.

² Published benomyl tolerances as of February 8, 1982.

³ pending tolerances now under review.

Increase of this tolerance to 4 ppm is being considered to include bound residues of the 5-hydroxy-MRC metabolite of benomyl.

Table 4

Maximum Single Serving Dietary Exposure for Benomyl

Food Commodity	Serving Size (kg)	Tolerance (mg/kg) ¹	Benomyl/MSC (mg/serving) - (mg/kg BW) ²
Apples*	0.212	7.00	1.48
Apricots*	0.114	15.00	1.71
Avocados	0.375	1.00	0.38
Bananas*	0.136	0.20	0.027
Beans*	0.072	2.00	0.14
Blueberries	0.145	7.00	1.02
Celery	0.160	3.00	0.48
Cherries*	0.145	15.00	2.18
Cucumber	0.144	1.00	0.144
Eggs	0.057	0.10	0.0057
Grapes	0.081	10.00	0.81
Grapefruit	0.121	10.00	1.121
Liver	0.120	4.00 ³	0.480
Mangoes	0.165	3.00	0.50
Meat, beef patty	0.113	0.10	0.011
Melons, cantaloupes	0.160	1.00	0.16
Melons, watermelon	0.160	1.00	0.16
Milk, cow	0.244	0.10	0.024
Mushrooms	0.095	10.00	0.95
Nectarines*	0.150	15.00	2.25
Nuts, cashew	0.140	0.20	0.028
Oranges	0.245	10.00	2.45
Peaches	0.174	15.00	2.63
Peanuts	0.152	0.20	0.03
Pears	0.180	1.00	1.26
Pineapple	0.155	35.00	5.43
Plums*	0.070	15.00	1.05
Poultry, chicken	0.140	0.10	0.014
Pumpkin	0.140	1.00	0.14
Raisins	0.142	50.00	7.10
Rice	0.100	5.00	0.50
Soybean oil	0.014	0.20	0.0028
Squash	0.222	1.00	0.222
Strawberries*	0.075	5.00	0.38
Tomatoes	0.181	5.00	0.91

*Thiophanate-methyl registrations also.

¹/ 40 CFR 180.294²/ 60 kg person³/ Increase of this tolerance to 4 ppm is being considered to include bound residues of the 5-hydroxy-MSC metabolite of benomyl (the current tolerance is 0.2 ppm).

Since the biological data did not suggest that a particular model was more appropriate than any other for risk estimation, the female MBC data were fit to several models (multi-stage, multi-hit, one-hit, probit, Weibull) and the results analyzed for goodness of fit as suggested by the Food Safety Council (1980). The multi-stage model provided the best fit. Lack of goodness of fit had a $p < 0.999$ indicating almost perfect fit of the data to this model (Litt, 1982a).

The potency estimator, Q^* , derived by the Agency using the multi-stage model was 2.065×10^{-3} (mg/kg body weight/day⁻¹). This is the slope of the lower 95% confidence bound on the virtually safe dose. Q^* is multiplied by the estimated exposure to obtain the upper 95% bound on the expected lifetime cancer risk from that exposure (Litt, 1982a).

The Agency's risk assessment is based on exposure data generated for benomyl use and an oncogenic risk estimator derived from data on liver tumor incidence in a two-year chronic feeding study in mice with the benomyl/thiophanate-methyl metabolite, MBC. Though not estimated directly, the oncogenic risk from thiophanate-methyl is assumed to be about the same as that from benomyl. This assumption is based upon their similar use patterns and data showing that both benomyl and thiophanate-methyl degrade to MBC in aqueous solution, in the soil, and in plants which are ingested by humans or domestic animals.

b. Oncogenic Risk from Estimated Dietary Exposure

The Agency calculated the oncogenic risk from potential dietary exposure to the benomyl and thiophanate-methyl metabolite, MBC, by using the potency estimator Q^* (2.065×10^{-3} mg/kg body weight/day⁻¹) and the dietary exposure estimates given in Table 3 of this document. The exposure and the upper limit of the risks are summarized in Table 5. Since thiophanate-methyl and benomyl have use patterns that are becoming increasingly similar and since both compounds appear to be broken down similarly in the environment and in animals, it is considered that the oncogenic risk presented by thiophanate-methyl would not exceed that presented by benomyl.

c. Oncogenic Risk from Use-Related Exposure

The Agency calculated the oncogenic risk to workers from exposure to benomyl by using the potency estimator (2.065×10^{-3}) given above and the user exposure estimates given in Table 2. The total lifetime individual risk estimates are presented in Table 6.

Table 5

Upper Bound on the Lifetime Oncogenic Risks Resulting
from Dietary Exposure to Benomyl and Thiophanate-methyl

Commodity	Exposure (mg/kg/day)	Upper Bound of Lifetime Risk*	Comments
Worst Case	0.03	7×10^{-5}	All crops treated and include MBC at tolerance levels. Includes a number of seed treatments and some pending tolerances. Excluding the seed treatments and the pending uses would only decrease the risk estimate to 6.6×10^{-5} .
TMRC adjusted to reflect percent of crop treated with benomyl	0.02	3×10^{-5}	Excludes seed treatments and pending uses.
Average Residue	0.003	7×10^{-6}	
Citrus Fruits	0.006 (adjusted for percent crop treated)	1×10^{-5}	Commodity with highest MBC contribution in the daily diet.
Soybeans	0.000001 (adjusted for percent crop treated)	2×10^{-9}	Commodity with lowest MBC contribution in the daily diet.

*Numbers are rounded.

Table 6
Oncogenic Risk Estimates for Workers and Users¹

Category	Total Exposure (mg/kg/yr) ²	Days Per Year	Lifetime Risk Without Mask	Lifetime Risk With Dust Mask
Aerial Application				
Grapes				
Mixer/loaders	0.35 (0.05)	5	4.9×10^{-6}	7.0×10^{-7}
Pilots	0.02	5	Dietary Background	Dietary Background
Flaggers	0.02	5	Dietary Background	Dietary Background
Fruit Crops				
Mixer/loaders	0.35 (0.05)	22	2.2×10^{-5}	3.1×10^{-6}
Pilots	0.02	50	Dietary Background	Dietary Background
Flaggers	0.02	50	Dietary Background	Dietary Background
Wheat				
Mixer/loaders	0.31 (0.05)	20	1.8×10^{-5}	2.8×10^{-6}
Pilots	0.02	20	Dietary Background	Dietary Background
Flaggers	0.02	20	Dietary Background	Dietary Background
Rice				
Mixer/loaders	0.26 (0.04)	10	7.4×10^{-6}	1.1×10^{-6}
Pilots	0.02	10	Dietary Background	Dietary Background
Flaggers	0.02	10	Dietary Background	Dietary Background
Soybeans				
Mixer/loaders	0.26 (0.04)	15	1.1×10^{-5}	1.7×10^{-6}
Pilots	0.02	15	Dietary Background	Dietary Background
Flaggers	0.02	15	Dietary Background	Dietary Background
Stone Fruits				
Mixer/loaders	0.26 (0.04)	40	2.9×10^{-5}	4.5×10^{-6}
Pilots	0.02	40	Dietary Background	Dietary Background
Flaggers	0.02	40	Dietary Background	Dietary Background
Berries				
Mixer/loaders/flaggers	0.22 (0.04)	22	1.4×10^{-5}	2.5×10^{-6}
Pilots	0.02	22	Dietary Background	Dietary Background
Air Blast Application				
Stone Fruits				
Commercial	0.03 (0.02)	40	3.4×10^{-6}	Dietary Background
Private	0.03 (0.02)	6	5.0×10^{-7}	Dietary Background

Table 2 (continued)

Category	Total Exposure (mg/kg day) ^{1,2}	Peak Conc. (mg/kg)	Exposure Risk (mg/kg day) With Diet Alone	Lifetime Risk With Diet Alone
Air Blast Application Continued				
Grapes				
Commercial	0.02	20	Dietary background	Dietary background
Private	0.02	10	Dietary background	Dietary background
Fruit Crops				
Private	0.02	10	Dietary background	Dietary background
Berries				
Commercial	0.02	100	Dietary background	Dietary background
Citrus	0.02 (0.02)	20	2.5 x 10 ⁻⁸	Dietary background
Ground Boom Application				
Turf	0.02	9	Dietary background	Dietary background
Wheat	0.02	10	Dietary background	Dietary background
Vegetables	0.02	5	Dietary background	Dietary background
Berries				
Private	0.02	20	Dietary background	Dietary background
Handspray				
Turf	0.02	9	Dietary background	Dietary background
Vegetables	0.02	5	Dietary background	Dietary background
Mushrooms	0.02	9	Dietary background	Dietary background
Homeuse	0.02	5	Dietary background	Dietary background
Reentry				
Adult	0.02	200	Dietary background	Dietary background
Minor	0.02	20	Dietary background	Dietary background

1/ Exposure data from Table 2.

2/ Exposure estimates for the use of dust masks (assumed to be 99% efficient) are shown in parentheses. All other estimates predominantly reflect dietary background exposure of 0.02 mg/kg day since the 100% dietary increment of exposure is lost in rounding after it is added to such exposure. These estimates were calculated by first subtracting the dietary increment of exposure, calculating 10% of the rest and adding back the dietary increment.

3/ Lifetime risks were estimated as follows:

$$\text{Risk} = (0^*) \times \left[\frac{\text{Working Life (Years)} \times \text{Dose (mg/kg day)}}{(\text{70 Year Lifetime}) \times (\text{mg/kg day})} \right]$$

$$0^* = 2.065 \times 10^{-3}$$

To calculate the lifetime individual risk, the Agency used the following formula:

$$\text{Risk} = (Q^*) \times \left[\frac{(\text{35 year working lifetime}) (\text{days exposed/year}) (\text{exposure})}{(\text{70 year lifespan}) (\text{365 days/year})} \right]$$

Thus, for example, the lifetime individual risk (R) for a mixer/loader from exposure to benomyl while mixing for aerial application to grapes is:

$$\begin{aligned} R &= (2.065 \times 10^{-3}) \times \left[\frac{(\text{35 years}) (\text{5 days per year}) (\text{0.35 mg/kg/day})}{(\text{70 years}) (\text{365 days/year})} \right] \\ &= \text{less than } 4.9 \times 10^{-6} \end{aligned}$$

The risks to users without respiratory protection (reflected in Table 6) ranges from less than 5.0×10^{-7} to 3×10^{-5} . The risk if a dust mask is used ranges from 7×10^{-7} to 5×10^{-6} . Risks are assumed to be similar for thiophanate-methyl since both it and benomyl have similar uses and metabolism.

2. Mutagenic Risk

The Agency issued a presumption based on suggested mutagenicity against benomyl on December 6, 1977 (US EPA, 1977a) and on thiophanate-methyl on December 7, 1977 (US EPA, 1977b). Both fungicides are metabolized to the active and presumed to have a role in their mutagenicity.

Studies specific for detection of gene mutation suggest that benomyl and PHE may be active. However, comparison of base lesions in the bacterial systems strongly suggest that the activity reported was initiated by contaminants of the active ingredients. This consideration is also applicable to integration of the lesions with the mouse lymphoma cell system. Consideration of lesions in the human DNA base and yeast gene conversion tests also strongly support a conclusion that benomyl and PHE do not directly interact with DNA, although these contaminants might do so.

Although benomyl has been shown to induce sister chromatid exchanges in Chinese ovary cells *in vitro*, studies with the same cell type showed no mutagenic activity at the HGPRT locus. Based on studies with the fungicide and its metabolite in cultures of rat and mouse liver cells, it is unlikely that the two chemicals induce DNA synthesis. In view of these findings and conditions of the SCE assay performed, the results cannot be considered as positive evidence that benomyl is capable of damaging DNA in Chinese hamster ovary cells. The other studies mentioned above suggest that the fungicide does not induce primary DNA damage.

Interference with cell division through binding to microtubulin potentially results in numerical chromosomal aberrations such as those detected in plants (tripolar anaphase nuclei, metaphase arrest, unbalanced chromosome number) and in animals (micronuclei, anaphase bridges, etc.). These effects are likely to result in teratogenicity in laboratory animals since morphological development of organs also depends on microtubules. Studies regarding these effects are discussed in the teratogenicity sections of this PD 4.

Studies cited by the Agency in PD 1 for benomyl (Bussey, 1968; Littlefield, 1969; and Hoffman and Kirsch, 1974) suggest that it is capable of reaching the gonads of experimental animals. However, the genotoxic effects discussed herein are more likely to arise from the effects on microtubules in dividing cells. Because there is no validated method for quantitatively assessing the hazards which may result from these effects, the significance of the presence of residues of benomyl or MBC in the gonads cannot be quantified (Gardner, 1982).

The Agency previously concluded in its PD 2 that thiophanate-methyl is not mutagenic. However, its metabolite MBC was considered to be a spindle poison. Available data and the absence of validated test procedures do not provide a basis for changing the previous conclusions as stated by the Agency in its Thiophanate-methyl PD 2, which indicated that the Agency does not have the means to estimate risk from such exposures.

3. Teratogenic Risk

Table 7 presents the results of benomyl teratology testing. These results show that teratogenic effects have occurred following gavage dosing of rats at levels ranging from 62.5 mg/kg/day to 500 mg/kg/day, and in mice at 250 mg/kg/day. The lowest NOEL determined by gavage dosing was 30 mg/kg/day (Scaples, 1980). Dietary dosing did not result in teratogenic effects in these studies with rats with doses from 5 to 500 mg/kg/day of benomyl or 500 mg/kg/day of MBC.

The Agency calculated margins of safety (MOS) for both dietary and non-dietary exposure to benomyl based on the provisional NOEL of 30 mg/kg/day. This was taken from the dietary teratogenicity study (Scaples, 1980) in which CAR-CD rats receiving a dose of 0, 5, 10, 30, 62.5 or 125 mg/kg/day of benomyl by gavage developed teratogenic effects at doses of 62.5 mg/kg/day and above. This study is being repeated by Dupont to confirm this NOEL, since biologically relevant eye effects were seen in two animals at 10 mg/kg/day; however, the incidence of this effect was not statistically significant at this level (Bochard, 1981b). This risk will be reassessed in the future if the results from Dupont's new study show a need to do so.

Table 8 presents the MOS's for non-dietary exposure to benomyl for teratogenic effects. These range from 86 to dietary background levels, assuming dietary exposure as reflected in Table 4 (single serving method of estimating peak dietary exposure). These MOS's are based on both the exposure calculated assuming no respiratory protection for benomyl users and respiratory protection in the form of a cloth or paper dust mask with which exposure to mixer/loaders could be reduced by up to 90% (EPA/OPP Protective Clothing Workgroup, 1982). This would then cause the teratogenic MOS's to range from 600 to dietary background levels if a dust mask were used during mixing/loading of benomyl.

Table 9 presents the dietary teratogenic risk MOS's based upon the single serving exposures discussed earlier in Section II.E.3. of this document. These MOS's range from 254 for raisins to 600,000 for soybean oil.

Teratogenic and Reproductive Effects of Phthalate

Species	Dosing	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
I. Teratogenic Effects				
A. Gavage Dosing				
Wistar Rats	1) 0, 62.5, 125, 250, 500 mg/kg/day during days 7-15 gestation. 2) as above, days 1-20 gestation gavage.	NOEL = 62.5	NOEL = 62.5	Schlenker and Turchinski, 1972
Wistar Rats	145 mg/kg, day 12 gestation gavage.	NOEL = 145		
Wistar Rats	0-500 mg/kg/day, days 6-15 gestation gavage.	NOEL = 1000 LOEL = 1000 Gestation termination observed	NOEL = 1000 (1000) LOEL = 62.5	Turchinski, 1973
CD-1 Mice	2000 or 4000 mg/kg/day in corn oil, days 6-15 of gestation gavage.	NOEL = 1000 LOEL = 200	NOEL = 1000 (1000) LOEL = 62.5	Sheet et al., 1979
CHR-CD Rats	0, 3, 10, 30, 62.5, & 125 mg/kg/day gavage.	NOEL = 30 LOEL = 62.5		Ravitsky, 1979
Sprague-Dawley Rat	Gavage-days 8-15 of gestation dose MRC=4.55 mg/kg dose benomyl=116 mg/kg	Small sample size precludes determination.		Staples, 1980
			Unilateral microphthalmia at 10 mg/kg/day in 2 animals. Embryotoxicity at 62.5 mg/kg/day above.	Delatour and Richard, 1976
			Terata produced-exencephaly (MRC treated) and other malformations	

Table 7 Continued

I. Teratogenic Effects Continued

A. Gavage Dosing Continued			
CD-1 Mice	0, 50, 100, 200 mg/kg/day on days 7-17 of gestation gavage	NOEL = 50 LEL = 100	Kavlock et al., 1982 All parameters affected at high dose. Weight, supra-occipital scars, supernumerary ribs, subnormal vertebral centra affected at 100 mg/kg/day.
Wistar Rat	0, 15.6, 31.2, 62.5, 125 mg/kg/day on days 7-16 of gestation gavage	NOEL = 31.2 LEL = 62.5	Kavlock et al., 1982 Embryonic resorptions at 125 mg/kg/day. Fetal weight reduced at 31.2 mg/kg/day and above skeletal maturity affected at 62.5 mg/kg/day and above. Detectable abnormalities at 62.5 mg/kg/day and above.
Wistar Rats	0, 15.6, 31.2 mg/kg/day from day 7 of gestation to day 15 of lactation gavage	NOEL = 15.6 LEL = 31.2	Kavlock et al., 1982 5% reduction in weight of testes and a 13% reduction in the weight of the ventral prostate and seminal vesicles at 31.2 mg/kg/day.
1. Dietary Dosing			
Charles River, CD Rats	5 mg/kg-400 mg/kg/day dietary through gestation day 15	NOEL = 400 (HMR) ¹ LEL = >400 (change)	Sherman, Cullik and Jackson, 1975 Not teratogenic.
CHR-CD Rats	Dietary MEC days 6-15 gestation; 0-10,000 ppm (500 mg/kg/day)	NOEL = 250 (5000 ppm) LEL = 375 (7500 ppm) (fetal weight)	Haskel Lab., 1978 Not embryotoxic or teratogenic. Slightly lower, not statistically significant fetal weights at 7500 ppm.
Wistar Rat	0, 125, 250, 500 mg/kg/day on days 7-16 of gestation-diet (calculated)	NOEL = >500 LEL = >500	Kavlock et al., 1982 No dose related incidence of anomalies/malformations in fetuses.

Table 7 Continued

II. Spermatogenic Effects

A. Gavage Dosing

Rats	>3400 mg/kg gavage (acute and subacute tests)	NOEL = <3400 LEL = 3400 (LDT)	Degeneration of germinal tissue and aspermatogenesis Testes = 10% target of benomyl (mature rats)	Sherman and Krauss, 1966
Rats	670 mg/kg (single dose) gavage (acute test)		Reduction of sperm (mature rats)	Sherman, 1965
Rats	10 doses, 200 mg/kg/dose gavage (subacute test)		Reduction of sperm (mature rats)	Sherman and Krauss, 1966
Wistar Rats	0, 15.6, 31.2 mg/kg/day on day 7 of gestation to day 15 of lactation gavage	NOEL = 15.6 LEL = 31.2	58% reduction in weight of testes and 13% reduction in weight of ventral prostate and seminal vesicles at 31.2 mg/kg/day	Kavlock et al., 1982
Sprague- Dawley Rat- Adult	0, 200, 400 mg/kg/day for 10 days gavage	NOEL = not determined LEL = 200	75-88% depression in total epididymal sperm counts & vas deferens sperm concentration at 200 or 400 mg/kg/day. Caudal weights depressed.	Carter and Laskey, 1982
Sprague- Dawley Rat- Adult	0, 400 mg/kg/day for 10 days gavage	NOEL = not determined LEL = 400	Slight to moderately severe hypospermatocytogenesis and slight to severe hypospermatogenesis	Carter and Laskey, 1982
Sprague- Dawley Rat- Prepubertal	0, 200 mg/kg/day for 10 days by gavage	NOEL = >200 LEL = >200	No damage to the reproductive system	Carter, Hein and Laskey, 1982

Table 7 Continued

II. Spermatogenic Effects Continued

B. Dietary Dosing

Dogs	0, 100, 500, 1,500, and 2,500 ppm Dietary chronic 2 year feeding*	NOEL = >2,500 ppm LEL = >2,500 ppm	No effect on sperm production (mature animals)	Sherman, Barnes and Stula, 1970
Rats	0, 100, 500 ppm Dietary chronic feeding	NOEL = >2,500 LEL = >2,500	No effect on sperm production (mature animals)	Sherman, Barnes and Stula, 1969
Rats (3 generation reproductive studies)	Up to 2,500 ppm in diet*	NOEL = 500 ppm LEL = 2,500 ppm (fetal weight decrease)	No effect on fertility index—no pathological changes attributable to MBC in diets—weights slightly decreased at 2,500–10,000 ppm in fetuses	Sherman, Culik and Jackson, 1975

C. Inhalation Dosing

Rats	Inhalation 33 mg/kg	NOEL = 7.5 LEL = 33	Reduction of spermatogenic activity (mature rats)	Hornberger, 1969
Dogs	Inhalation 82 mg/kg	NOEL = 32 LEL = 82	Reduction of spermatogenic activity (mature dogs)	Littlefield, 1969

*MBC fed in diet

1/

LEL = lowest effect level
 NOEL = no observed effect level
 LDT = lowest dose tested
 HDT = highest dose tested

Table 8

Teratogenic and Spermatogenic Risk Estimates for Benzyl Exposure to Users¹

Category	Hours Per Day	Total Exposure (mg/kg/day) ²	Teratogenic Effects MOS ³		Spermatogenic Effects MOS ³	
			Without Dust Mask	With Dust Mask	Without Dust Mask	With Dust Mask
Aerial Application						
Grapes/Fruit Crops						
Mixer/Loaders	8	0.35 (0.05)	86	600	21	150
Pilots	3	0.02	Background	Background	Background	Background
Flaggers	8	0.02	Background	Background	Background	Background
Wheat						
Mixer/Loaders	7	0.31 (0.05)	97	600	24	150
Pilots	3	0.02	Background	Background	Background	Background
Flaggers	7	0.02	Background	Background	Background	Background
Rice/Soybeans/Stone Fruits						
Mixer/Loaders	6	0.26 (0.04)	115	750	29	188
Pilots	3	0.02	Background	Background	Background	Background
Flaggers	6	0.02	Background	Background	Background	Background
Berries						
Mixer/Loaders	5	0.22 (0.04)	136	750	34	188
Pilots	3	0.02	Background	Background	Background	Background
Air Blast Application						
Stone Fruits						
Commercial	7	0.03 (0.02)	1,000	Background	250	Background
Private	8	0.03 (0.02)	1,000	Background	250	Background
Grapes						
Commercial	3	0.02	Background	Background	Background	Background
Private	4	0.02	Background	Background	Background	Background
Fruit Crops						
Private	6	0.02	Background	Background	Background	Background
Berries						
Commercial	3	0.02	Background	Background	Background	Background
Citrus	8	0.03 (0.02)	1,000	Background	250	Background

Table 8 Continued

Category	Hours Per Day	Total Exposure (mg/kg/day) ²	Teratogenic Effects MYS ³		Spermatogenic Effects MYS ⁴	
			Without Dust Mask	With Dust Mask	Without Dust Mask	With Dust Mask
Ground Room Application						
Turf	6	0.02	Background	Background	Background	Background
Berries Private	3	0.02	Background	Background	Background	Background
Wheat	8	0.02	Background	Background	Background	Background
Home Use	0.05	0.02	Background	Background	Background	Background
Mushrooms	4.5	0.02	Background	Background	Background	Background
Turf-Handspray	0.5	0.02	Background	Background	Background	Background
Vegetables	2	0.02	Background	Background	Background	Background
Reentry Adult	7.5	0.02	Background	Background	Background	Background
Minors	7.5	0.02	Background	Background	Background	Background

1/ Exposures from Table 2. This estimate basically reflects inhalation exposure to benomyl assuming 100% absorption of respired particles added to the calculated average annual dietary exposure of 0.02 mg/kg/day (a small increment has been added to reflect dermal penetration for mixer/loaders only). Exposure from post harvest use on citrus, use on pineapples and sugar cane is negligible due to apparatus associated with use.

2/ Exposure estimates for the use of dust masks (estimated to be about 90% effective) are shown in parentheses. All other estimates predominantly reflect dietary background exposure of 0.02 mg/kg/day, since the work related increment of exposure is lost in rounding after it is added to such exposure.

3/ MYS = (NOEL of 30 mg/kg/day)/(Exposure); from Staples (1980) gavage study in rats. Background indicates that the work-related exposure increment is negligible when compared to the annualized average dietary exposure of 0.02 mg/kg/day.

4/ MYS = (NOEL of 7.5 mg/kg/day)/(Exposure); from Hornberger, (1969) inhalation study in rats. Background indicates that the work-related exposure increment is negligible when compared to the annualized average dietary exposure of 0.02 mg/kg/day.

Table 9
Single Serving Dietary MDS's for Renomyl's Potential Teratogenic and Spermatogenic Effects¹

Food Commodity	Renomyl/MPC (mg/serving) - (mg/kg BW)	Teratogenic Effects MDS	Spermatogenic Effects MDS
Apples	1.48	1,200	300
Apricots	1.71	1,040	260
Avocados	0.38	4,760	1,190
Bananas	0.027	60,000	15,000
Beans	0.14	12,500	3,130
Blueberries	1.02	1,770	440
Celery	0.48	3,620	900
Cherries	2.18	830	210
Cucumber	0.144	12,500	3,100
Eggs	0.0057	300,000	75,000
Grapes	0.81	2,140	540
Grapefruit	1.121	1,500	380
Liver	0.480	3,750	900
Mangoes	0.50	3,750	900
Meat, beef patty	0.011	150,000	37,500
Melons, cantelopes	0.16	11,110	2,700
Melons, watermelon	0.16	11,110	2,700
Milk, cow	0.024	75,000	18,800
Mushrooms	0.95	1,880	470
Nectarines	2.25	790	200
Nuts, cashew	0.028	60,000	15,000
Oranges	2.45	730	180
Peaches	2.63	680	170
Peanuts	0.03	60,000	15,000
Pears	1.26	1,430	360
Pineapple	5.43	330	83
Plums	1.05	1,670	417
Poultry, chicken	0.014	150,000	37,500
Pumpkin	0.14	13,040	3,300
Raisins	7.10	250	64
Rice	0.50	3,620	900
Soybean oil	0.0028	600,000	150,000
Squash	0.222	8,110	2,000
Strawberries	0.38	4,760	1,200
Tomatoes	0.91	2,000	500

1/
60 kg person

It should be noted that the procedure of using results of animal tests employing gavage dosing (as was done by Staples, 1980 from which the 30 mg/kg/day NOEL was derived) to calculate human risk from dietary exposure is controversial. DuPont (Smith, 1981) considers such risk estimates to be inappropriate, preferring instead to use results from studies employing dietary dosing of animals. DuPont points out, that gavage studies with benomyl have induced teratogenic effects at doses of 62.5 mg/kg/day and greater. Dietary dosing on the other hand, has not induced teratogenic effects.

Further, DuPont maintains that benomyl and MBC can cross the placenta following gavage administration to rats (Haskell Laboratory, 1980a), but with dietary administration, benomyl does not cross the placenta in detectable quantities (Haskell Laboratory, 1980b). DuPont considers that a large "bolus" dose of the compound administered by gavage might overwhelm the metabolic capacity of the liver, resulting in high doses of benomyl to the embryo. Large dietary doses, however, are more slowly absorbed and can be rapidly metabolized by the liver, giving low maternal blood levels and no detectable embryonic levels of benomyl. They concluded that dietary administration was a more appropriate method for human hazard assessment.

The FIFRA Scientific Advisory Panel (SAP) has acknowledged the contradictory results obtained from the two methods of dosing and have recommended that any NOEL established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in dietary studies (Fowler, 1979).

Kavlock et al. (1982) compared dietary and gavage administration in rats and mice. Oral administration was teratogenic above 31.2 mg/kg/day in the rat and above 50 mg/kg/day in the mouse. Dietary administration caused only fetal growth retardation in rats at the highest test dose (505 mg/kg/day). Kavlock et al. (1982) pointed out that rats consume most of their feed in short bouts throughout the night with some being consumed during the day. Assuming equal metabolic potential and absorption into the blood, dietary administration of chemicals like benomyl with short half-lives would result in low circulating levels in the blood since excretion per unit of time can equal or exceed ingestion per unit time. Since humans consume most of their food at two to three discrete daily time periods, higher peak plasma levels of benomyl would be expected from the human feeding pattern than would occur in the rat. Kavlock et al. (1982) concluded that for chemicals like benomyl which have short biological half-lives, gavage is the appropriate dosing regimen for basing human risk extrapolations from dietary exposure.

The Agency considers that gavage administration of test material is scientifically more acceptable than dietary dosing for determining a NOEL for teratogenicity because it eliminates problems of palatability, drug stability, nutrient integrity and calculations of accurate dose levels. The Agency supports the Kavlock et al. (1982) opinion, believing that gavage assures relevance of treatment to the human condition for chemicals like benomyl, since rodents, by preference, eat frequently during waking hours, whereas humans dine at relatively orderly intervals during the day. Therefore, the peaking of

blood residues following gavage administration more clearly parallels the human situation than continuous uptake of the chemical in the rat diet. Even so, the Agency considers that gavage may be a more conservative indicator of this risk and in interpreting MOS's the Agency has taken this into consideration.

4. Risk from Spermatogenic Effects in Males

Table 7 presents the results of benomyl/MBC spermatogenic effects testing. These results show that spermatogenic effects have occurred following both single and multiple gavage dosing of rats at dose levels from 62.5 mg/kg/day to 3400 mg/kg/day. The lowest NOEL determined by gavage dosing (both during gestation and lactation) was 15.6 mg/kg/day (Kavlock, Chernoff and Gray, 1982). Inhalation dosing in dogs and rats caused a reduction of spermatogenic activity in rats at 33 mg/kg/day (Hornberger, 1969) and in dogs at 82 mg/kg/day (Littlefield, 1969). The NOEL determined by inhalation testing was 7.5 mg/kg/day. The next highest dose level was 33 mg/kg/day (Hornberger, 1969). Dietary dosing of dogs and rats at levels greater than 2,500 ppm did not affect sperm production. Further, the fertility index of rats was not affected by dietary dosing in a three-generation reproductive effects study at levels up to 2,500 ppm.

The Agency calculated margins of safety (MOS) for both dietary and non-dietary exposure to benomyl based on the NOEL of 7.5 mg/kg/day developed in the inhalation study (Hornberger, 1969). Non-dietary MOS's are shown in Table 8. The MOS's range from 21 to dietary background levels for benomyl users without respiratory protection. Exposure for mixer/loaders could be reduced by up to 90 percent by use of a dust mask (EPA/OPP Protective Clothing Workgroup, 1982). This would cause the spermatogenic effects MOS's to range from 150 to dietary background levels if a dust mask were used during mixing/loading of benomyl.

Table 9 presents the dietary MOS's for spermatogenic effects based upon the single serving exposures discussed in Section II.E.3. of this document and the NOEL of 7.5 mg/kg/day (Hornberger, 1969). These range from 64 for raisins to 150,000 for soybean oil, if tolerance levels are assumed to be present in the treated commodities.

G. Aquatic Hazard

In PD 1, the Agency presumed against benomyl products which are registered for direct application to water (rice), because the calculated residue of benomyl in water after direct application (734 ppb) is greater than one-half the acute LC_{50} for channel catfish (8-12 ppb), bluegill sunfish (0.4-1.2 ppm) and Daphnia magna (0.64 ppm) (U.S. EPA, 1977a).

In PD 2/3 (US EPA, 1979a), the Agency concluded that the presumption of aquatic risk to catfish had not been rebutted. In so doing, the Agency rejected the contention of DuPont that benomyl was not registered for any uses involving direct application to water, that no aquatic organisms are exposed to benomyl, that use according to label directions would not likely cause significant adverse effects, that no fish kills have resulted from benomyl use according to

label directions, that actual application to rice fields does not exceed one-half the LC_{50} for bluegill sunfish or Daphnia, and that significant amounts of benomyl do not move downstream from rice fields.

The PD 2/3 concluded that benomyl's use on rice posed a significant hazard to natural, local populations of channel catfish, other Ictalurids, and fish food organisms. The Agency also concluded that aerial use on berries and orchards may pose a threat to sensitive aquatic species. Since available information was insufficient to quantify this risk, the Agency proposed to require additional field monitoring studies for benomyl. These would involve monitoring in replicated fields treated twice at 1/4, 1/2, and 1 pound active ingredient per acre with residue samples collected before and after application, and after drainage (in rice fields, main drainage ditches, nearest body of water at the entry point and several places downstream).

The Agency has reconsidered the scope of this study and now has determined that monitoring as described above will only be required for benomyl at typical application rates. Registrants will be provided with general guidelines for this study by the Agency. Registrants should meet with the Agency to develop final details of protocol for this study.

III. Qualitative Benefits Analysis

A. Benomyl

The Agency conducted an economic impact analysis of benomyl use on apples (pre- and post-harvest), citrus (pre- and post-harvest), grapes (including raisins), pineapples, stone fruits (pre- and post-harvest) and rice. These sites had been preliminarily identified as benomyl use sites that were likely to present the greatest risk in terms of teratogenicity, oncogenicity, and non-target aquatic organisms. Information was gathered from within the Agency and from the USDA/States/EPA Benomyl Assessment Team report. Total economic impacts associated with a cancellation action on these sites would exceed a range of \$26 to \$52 million per year (Gaede, 1982a).

1. Benomyl Usage

Total US usage of benomyl was estimated to range from 2.5 to 3.0 million pounds active ingredient per year (Table 10) and represents about 2 percent of the total US fungicide market (US EPA, 1980). The largest volume use site accounting for approximately 100,000 pounds active ingredient or more were: apples, citrus, grapes, pecans, stone fruits, rice, soybeans, ornamentals, turf and vegetables. The sites covered by this analysis have a total annual usage ranging from 0.81 to 1.03 million pounds active ingredient (about one-third of the total US benomyl market).

2. Apples

Annual pre-harvest usage of benomyl on apples ranged from 75,000 to 187,500 pounds active ingredient applied on 90,000 to 150,000 acres (18 to 31 percent of the US apple orchard acreage). For post-harvest treatments, 10,000 pounds active ingredient benomyl are annually applied to 2.02 million tons or 50 percent of US apple production (Table 10).

a. Pre-harvest Apples

Benomyl alternatives for pre-harvest use generally provide good to excellent control with captan, dodine, maneb plus dinocap, and captafol being widely used. Existing alternatives are adequate for many disease problems, but under heavy disease pressure, economic losses would increase if benomyl were not available. For growing seasons with low to moderate disease pressure, economic impacts to the growers would be limited to the increased cost of alternative controls. In this case the annual production cost increases would be expected to range from \$0.3 to \$0.8 million. During growing seasons with heavy disease pressure (i.e. wet seasons), both increased treatment costs and production losses would be expected if benomyl were not available; increased treatment costs would annually range from \$1.4 to \$2.7 million and the value of lost production would be expected to range from \$1.8 to \$3.0 million for each additional one percent loss in impacted production. Consumer impacts would only be expected in years with heavy disease pressure and output losses.

Table 10. Summary of the Economic Importance of Rotenone^a

Site	Extent of Use		Quantity Rotenone Applied (Lbs. Active Ingredient)	Availability of Economic Alternatives	Economic Impact
	Units Treated	% of U.S.			
Fruits and Nuts					
Apples, Preharvest	90,000-150,000 acres	18-31	75,000-187,500	Captan, dichlorone, dodine, sulphur, captan, dinocap ferbam, maneb + dinocap	Minimum of \$.3-.8 million per year in dry years. \$1.4-2.7 million in wet years plus production losses (\$1.8-3.0 million for every 1% loss). Minor consumer impacts.
	Postharvest: 2.02 million tons	50	10,000	TMG	\$104,000/year plus losses due to increased storage rot (\$6.25 million for each 1% loss).
Citrus, Preharvest					
	Oranges	20	120,000	Oil, copper, captan	\$1.4 million total citrus; mostly fruit downgrading and increased shipping losses of grapefruit for the Japanese market.
	Grapefruit	18	52,500		
Other	100,000 acres	6	8,500		
Postharvest	7.9 billion lbs.	26	5,000 - 6,000	TMG is the major alter- native; prop + diphenyl often used on exported fruit.	\$606,000 per year but in- creased losses due to decay in shipping and marketing. Decay loss un- known but each 1% loss = \$4.6 million. Some retail price increases and home fruit decay problems.
Grapes	100,000-125,000 acres	12-16	70,000-110,000	Captan, folpet, maneb, copper, dieldrin	\$1.4-3.9 million per year plus production losses, especially in wet growing seasons. Production los- ses would increase the retail prices for grapes and raisins.
Pineapples	10,770 tons	15% of fresh crop in Hawaii	NA (Not Available)	Captan and prop for fresh fruit; captan and captan for planting stock	\$1.92 million loss in pro- duction. 2.1% reduction in HI crop, further loss of 1% of fresh supply. Retail price increases for fresh and canned fruit.

Table 10 Continued

Site	Extent of Use		Quantity (lb/acre) Applied (Lbs. Active Ingredient)	Availability of Economic Alternatives	Economic Impact
	Units Treated	% of U.S.			
Stone Fruits					
Prunus					
Peaches	150,000-200,000 acres	50-75	112,500-187,500	Captan, sulphur, morph (captan), dodine	3.39-6.57 13.11-19.07 0.3-0.45 1.28-1.88 2.08-2.24 4.93-5.82 Production losses in wet season would increase retail prices
Nectarines	9,200-13,600 acres	50-73	7,400-11,600		
Cherries	40,000-50,000 acres	34-42	37,500-41,250		
Postharvest					
	NA	NA	NA	Dichloran	Not quantified. Current losses of 2 to 3% per year (56-9 million) would be exceeded by an unknown amount. Probable retail price increases (amount unknown).
Almonds					
	NA	NA	63,800	NA	NA
Avoicados					
	310 acres	5 (PL)	1,600	Copper (poor alternative)	NA
Bananas					
	NA	Not grown in US	200,000 (Central America)	Mancosb, morph thiophanate methyl	NA
Berries					
	NA	Blueberries East 90 West 50	71,911	Perham, captan, lime sulphur, dinocap	NA
Macadamia nuts					
	3,200 acres	80	100 - 2,400	Difolatan provides equivalent control	NA
Mangos					
	800 acres	50 (PL)	6,000	Copper is less effective	NA
Pears					
	NA	NA	10,928	Dodine, dinocap, sulfur	NA
Pecans					
	100,000 acres	20	100,000	Dodine and triphenylethyn hydroxide	NA

Table 10 Continued

Site	Extent of Use Units Treated % of U.S.	Quantity Benzoyl Applied (Lbs. Active Ingredient)	Availability of Economic Alternatives	Economic Impact
<u>Field Crops</u>				
Peanuts	332,000 acres	22	158,500	Chlorothalonil, maneb, difolatan mancozeb, triphenyltin hydroxide coppers, others
Rice	442,300 acres	22	299,300	None
Soybeans	1,522,000 acres	2.6	781,000	Thiabendazole
Sugar Beets	NA	NA	13,500	NA
Sugar Cane- field pieces	NA	NA	26,000	None
Wheat	NA	NA	11,150	None
<u>Ornamentals</u>				
Ornamentals	NA	NA	125,000	Dinocap, chlorothalonil, captan fifeth, maneb, thiram, thiophanate methyl, folpet, mancozeb, coppers, sulphur, metiram, nabam, others
Turf	NA	NA	150,000	Thiophanate, thiophanate-methyl, cadmium, cycloheximide, POB, chlorothalonil, thiram, mancozeb, others
<u>Vegetables</u>				
Mushrooms	174 million lbs.	50	11,034	Fifeth
Vegetables	NA	NA	158,500	Folpet, maneb, chlorothalonil, dinocap, captan, anilazine, others
TOTAL		2.47 to 2.71 million		Greater than \$26.37 million to \$52.34 million.

* Source: USDA (1976), USDA/US EPA (1979), US EPA (1980), US EPA (1981).

- 1/ Limited to domestic usage only. Benzoyl use for Central American banana production is not considered in the overall total.
- 2/ Aggregated economic impacts are for the production/processing levels of the market and are limited to the following: peaches, apples (pre- and postharvest), citrus (pre- and postharvest), grapes, pineapples, stone fruits (pre- and postharvest), and rice.

b. Post-harvest Apples

TBZ is the major post-harvest treatment alternative for benomyl on apples. The total increase in annual treatment costs for TBZ relative to benomyl was estimated as \$104,000 or about \$0.05 per ton of apples treated. Though storage losses would be likely if this alternative were used, the total value of lost output could not be accurately projected. However, for each additional one percent loss of stored apples, a loss of at least \$6.25 million would be expected.

Consumer impacts would be related to the reduced storability of apples and the decreased retail supplies during the spring and summer months. Fresh apple prices received by producers near the time of harvest would be expected to decline by some unknown magnitude if benomyl were not available. With reduced fresh apple supplies during the spring and summer, retail prices would then be expected to increase by some unknown degree.

3. Citrus

Annual pre-harvest usage of benomyl on citrus from Florida was estimated to be about 181,000 pounds applied to 205,000 acres (16 percent of the US citrus orchard acreage). Benomyl as a post-harvest treatment accounted for 5,000 to 6,000 pounds active ingredient per year for about 7.9 million tons (26 percent) of US citrus production (Table 10).

a. Pre-Harvest Citrus

Benomyl alternatives for greasy spot include copper compounds or petroleum oils; copper is avoided for use on fresh market fruit as well as on 50 percent of the Florida citrus acreage where phytotoxicity may be a problem. Captafol is an effective control for scab but can only be used when citrus trees are dormant.

The short run annual economic impact to Florida growers would be about \$1.4 million for increased treatment costs of alternatives, fruit downgrading, and a 10 percent increase in grapefruit shipping losses to Japan. For years with heavy disease pressure, the economic impact upon growers would be higher because of an anticipated increase in fresh fruit downgrading.

Consumers would be impacted by the reduced quality of fresh citrus (particularly during years of heavy disease pressure) as well as by reduced quantities supplied to the retail market. Retail price increases of an unknown magnitude would be expected.

b. Post-Harvest Citrus

As a post-harvest treatment TBZ would be the primary alternative for benomyl on citrus; SOPP and diphenyl would frequently be used in combination as a treatment for exported fruit. The increased treatment cost of using TBZ as a replacement for benomyl was estimated as \$606,000 with \$370,000 of this impact occurring in California. These production cost impacts are best case

estimates, since disease organism resistance problems with TBZ would prevent a total substitution for benomyl.

The value of output losses during storage or shipping could not be estimated with available data, but are potentially very significant. For each one percent additional loss for stored or shipped fruit, a \$4.6 million impact would be expected.

Consumers would probably incur a higher rate of fruit storage loss at-home as well as increased retail prices.

4. Grapes

The total annual usage of benomyl on grapes ranges from 78,000 to 110,000 pounds active ingredient (Table 10). Benomyl was applied to 100,000 to 125,000 acres of grapes which represents 12 to 16 percent of the total US grape acreage. In addition, 31,000 to 39,000 acres (4 to 5 percent of the US acreage) of grapes intended for raisins were also treated with benomyl.

Captan, folpet, copper compounds, dicloran and maneb are as effective as benomyl under some growing conditions, but generally require multiple applications. However, disease induced losses associated with wet growing seasons (50 percent of the seasons) would be expected to increase without benomyl.

Under dry growing season conditions, a \$1.1 to \$3.9 million grower impact would be expected because of higher control costs with alternatives. For wet growing seasons, grower impacts would increase because of output losses. For each 5 percent incremental loss in output, grower economic impacts would increase by \$9.5 to \$11.9 million.

Consumers would be impacted by higher retail prices if output losses occur. Retail price increases would be expected, but are difficult to estimate because of variability in disease incidence and pressure.

5. Pineapples

Total annual usage of benomyl to treat either pineapple seed pieces or fresh pineapple is not known. Estimates of benomyl treatments included enough seed pieces to plant about 5,000 Hawaiian pineapple acres as well as 10,770 tons (15 percent of Hawaiian production) of fresh fruit (Table 10).

Captan and captafol are registered for use on seed pieces but captan does not provide acceptable control of Thielaviopsis and captafol is not used because of allergic reactions by some workers. Captan and SOPP are registered for use on fresh fruit but are less effective than benomyl under high spore loads.

Grower impacts associated with seed piece treatments were estimated as \$1.67 million for about 14,200 tons of reduced output. Shipping losses of fresh pineapple were estimated as 754 tons valued at about \$0.26 million. Therefore, the total annual industry impact would be about \$1.92 million.

Reduced Hawaiian production (2.1 percent) and fresh market fruit shipping losses (about 1 percent) would decrease the quantity of retail supplies. Such retail supply reductions would be expected to increase consumer prices for both fresh and processed pineapple products.

6. Stone Fruits

The annual usage of benomyl as a pre-harvest disease control on stone fruits accounted for 157,400 to 240,350 pounds active ingredient that were applied on 199,200 to 263,600 acres (Table 10). The total extent of benomyl usage as a post-harvest treatment of stone fruit is not known; approximately two thirds of California's fresh peaches are treated and usage levels for other stone fruits are also believed to be extensive.

Dicloran is a registered alternative that provides excellent control of Rhizopus rot but has only modest activity against brown rot. Smooth skinned stone fruits such as cherries and nectarines do not load sufficient dicloran to prevent brown rot.

Growers and packers of stone fruits would utilize dicloran to whatever extent possible. Increased losses of fruit would be expected because of a higher incidence and severity of fruit rots during shipping and marketing; the magnitude of these increased losses cannot be estimated with available data.

Consumers would likely experience price increases of unknown magnitudes for the reduced marketings of fresh stone fruits.

7. Rice

The annual usage on rice was estimated as about 299,300 pounds active ingredient applied to 422,300 acres (22 percent of the US acres) (Table 10). No other fungicides are registered as alternative controls.

Without benomyl, the annual income loss to impacted growers was estimated to be about \$15.0 million after benomyl treatment cost savings (\$7.8 million) are deducted from yield losses (\$17.8 million) and quality losses (\$5.0 million). On a per acre basis, the farm level impact ranged from \$23 to \$28 per year.

Little or no consumer level impacts would be expected if benomyl were not available for use on rice.

B. Thiophanate-Methyl

The Agency has not quantified the benefits of thiophanate-methyl use since most product registrations have been in effect for less than two years. However, if equivalent efficacy with benomyl is assumed based upon both products fungitoxic action being associated with a common metabolite, MBC, then thiophanate-methyl would have the potential for achieving larger benefits than are now associated with its new uses.

Given the larger differences in total acre treatments, the economic importance of thiophanate-methyl is now exceeded by that of benomyl. If the future site registrations for benomyl and thiophanate-methyl continue to become more correspondent, and if they are equally efficacious, these two fungicides will become better economic substitutes for one another. With improved economic substitutability, increased competition would be expected to affect the relative market shares of these two fungicides.

The future economic importance of either thiophanate methyl or benomyl relative to one another can not be easily determined, since outcomes would be affected by the success of each manufacturer's marketing strategy and the final user's perception of any competitive advantage existing between the two products. In aggregate, the joint economic benefits of thiophanate-methyl and benomyl are currently large; any future price reductions due to increased market competition would be expected to increase both overall usage and economic importance by some unknown magnitude as long as both products continue to be effective (Gaede, 1982b).

IV. Analysis of Comments

After the preliminary Notice of Determination was issued on benomyl and thiophanate-methyl (August 30, 1979 and October 3, 1979 respectively), the Agency received comments from the Secretary of Agriculture, the Scientific Advisory Panel (SAP), DuPont, and other concerned individuals and organizations. The responses to comments on the benomyl PD 2/3 are organized by topic and are discussed below. As indicated in the following discussion, some aspects of the PD 2/3 risk assessment and regulatory requirements have been changed to reflect the recommendations of the SAP and others.

A. Comments Relating to Risk

1. Benomyl

a. Mutagenicity Testing

In response to the Agency's request that additional gene mutation tests using benomyl be carried out in Drosophila, mammalian somatic cells in culture and in an appropriate eukaryotic microorganism, the SAP referred the Agency to its statements of May 31, 1978 that recommended a battery of tests for general mutagenicity screening (Fowler, 1979) which would include a sophisticated microbiological test such as an enhanced Ames assay with appropriate dose response; a mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma; and an in vivo cytogenetic assay and a multiple generation test as part of a reproduction study modified to provide a statistically significant assay for dominant lethal mutations. The SAP also recommended that oncogenicity studies be used in the overall evaluation of mutagenicity.

SAP stated that the requirement for a sophisticated microbiological test using benomyl had been met and that sufficient in vivo cytogenetics tests had also been completed. Since it appeared that an appropriate mammalian cell point mutation assay had not been carried out using benomyl, SAP recommended that such an assay be performed using either a mouse lymphoma or a Chinese hamster embryo cell line or another equally well validated mammalian cell test for point mutation. SAP stated that no further mutation testing should be required and the mutation trigger should be considered to have been completely rebutted, if the results of that assay were negative.

With regard to the recommended test battery, the Agency considers the results from mammalian point mutation assays with benomyl to be uncertain and in need of confirmation. The existing mouse lymphoma study showed a weakly mutagenic response (mutation frequency 2 to 3 times that in the control culture) at a dose which killed about 80 percent of the test cells. Benomyl and MBC did not cause mutations in Chinese hamster ovary cells in vitro. Data suggest that positive results noted in certain point mutation studies could be due to contaminated test material. Study results on the MBC metabolite of benomyl and thiophanate-methyl are negative for point mutagenicity (Gardener, 1982).

Data indicate that benomyl and MBC do not directly interact with DNA, although their contaminant might do so. Dominant lethality was not demonstrated in an existing test with benomyl (Gardener, 1982).

An in vivo cytogenetics test with benomyl showed an increased frequency of polychromatic erythrocytes with micronuclei in mice given up to 1,000 mg/kg doses of benomyl. However, the preponderance of data suggest that the observed micronuclei are more likely the result of spindle effects (microtubule inhibition during cell division) rather than direct chromosome damage (Gardener, 1982).

b. Spindle Effects

1) Spindle Effects Testing

SAP indicated that the best methods for predicting adverse health effects in man from spindle poisons were the multigeneration reproductive test and the dominant lethal test. The multigeneration study modified in this way was recommended as the primary means by which the Agency should estimate the potential risk to man from spindle poisons. SAP stated that if this was negative that cytogenetics be conducted on the sperm and bone marrow cells of an adequate number of the animals used in this and other studies. SAP also urged the Agency to fund research with the aim of developing the amniocentesis rodent model and the in vivo test in mammalian cells for spindle effects, as well as research on the evaluation of the predictability of in vivo tubulin binding of spindle poisons.

The Agency differs with the SAP in that the Agency does not consider the in vivo tests recommended by the Panel (multigeneration reproductive, dominant lethal and cytogenetics) sufficiently sensitive to adequately assess risk from spindle inhibitors (Mauer, 1980). The Agency does agree, however, with the desirability for the development of sensitive in vivo assays for spindle effects, as well as the funding of research on the predictive value of tubulin binding. The Agency cannot suggest at this time an alternative set of tests, since none of the assay systems currently proposed for use in screening programs can unequivocally detect chemicals producing spindle effects significantly relevant to man, such as aneuploidy by nondisjunction or any other mechanisms. The following is a portion of a summary statement by OPP staff scientists delineating the position of the Agency in this regard (Hill, 1980):

"The Scientific Advisory Panel (SAP) submitted to the Office of Pesticide Programs (OPP) its evaluation of chemicals which may interfere with the cell-division spindle (benomyl, thiophanate-methyl and trifluralin) on November 30, 1979 (Appendix A). The SAP is willing to accept data from a few test systems which are presently available (reproduction and dominant lethal tests plus in vivo cytogenetics) to evaluate safety in regard to spindle effects. Using this scheme the Panel concluded that adequate testing has been done for benomyl in this regard.

On the one hand, OPP agrees with the Panel that nothing in the review of all the information available on these chemicals, including subchronic toxicity and reproduction studies, indicated significant adverse effects from this toxicity. On the other hand, OPP does not think these systems have the capability to assess fully the degree of hazard from spindle inhibition.

Basically, OPP staff is of the opinion that there is a lack of information bearing on the sensitivity and adequacy of various tests to evaluate risks from spindle inhibition and, more generally, inhibition of microtubular protein polymerization.

In addition to in vivo tests, the Panel also suggested research funding for the development of in vivo assays for spindle effects of pesticides, contaminants and breakdown products (such as examining metaphase arrest in mammalian cells), as well as approaches to evaluate the predictability of in vivo tubulin binding of spindle poisons.

The OPP staff agrees with the SAP as to the need for further investigation on the usefulness of various systems for evaluating risks. To this end, the Agency plans to identify outside scientists who can help us delineate a meaningful research program to fill some of the information gaps and to summarize possible means of risk assessment.

The problem of assessing risks is heightened when one considers on a broader scale the development of schemes for chemicals which inhibit microtubular polymerization. In this case, one is concerned not only with spindle effects but also all other effects produced by chemicals acting via the molecular mechanism of microtubular inhibition. Since these inhibitors affect all microtubule-related processes, including cell shape, cell movement, intracellular molecular movement, and cellular secretion, many functions may be affected by these 'cellular poisons.' Given these effects, we must decide what are the appropriate toxicological endpoint of concern, how to evaluate them and, lastly, how to evaluate risks. Because of the magnitude of the problem we have decided to start with spindle effects and to enlarge the scope of problems over time."

2) Spindle Effects Threshold

SAP also stated that it believed a threshold existed for spindle effects from compounds such as benomyl. Considering the level of exposure to benomyl (mixer/loaders and consumers via the diet) SAP was of the opinion that an adequate margin of safety existed for the potential spindle effects of benomyl in man. In addition SAP was of the opinion that there was no significant risk from spindle inhibition in man as a result of exposure to thiophanate-methyl in its normal use in agriculture.

DuPont (Bucknum, 1979) stated that although benomyl is a mitotic spindle inhibitor, heritable effects resulting from this mechanism have not been demonstrated. DuPont questioned the Agency conclusion that benomyl might be capable of inducing the failure of chromosomal separation at anaphase thereby leading to numerical chromosomal aberrations such as aneuploidy. DuPont indicated that non-disjunction induced by benomyl has not been demonstrated in any mammalian cells--germ or somatic. In fact, definitive evidence of aneuploidy induced by non-disjunction has not been demonstrated in any cell and that aneuploidy is not a consequence of mitotic inhibition in whole animals. DuPont stated that since a heritable effect had not been demonstrated that this mutagenicity presumption has also been rebutted.

DuPont also stated that a margin of safety could be calculated for this effect. DuPont cited statements at the April 1979 SAP meeting by Dr. Wilson (above) and Dr. Chaisson (EPA) which indicated that there appears to be a threshold for this type of effect and a no-observable effect level for benomyl of 500 mg/kg.

Dr. Leslie Wilson (3(30000/23B) took issue with the Agency's statement that it did not have the means to establish the degree of risk for spindle effects. Dr. Wilson suggested that benomyl's antimitotic action appeared to be due to its tubulin-binding ability. Benomyl binds to tubulin at the same site as colchicine, although benomyl's potency appears to be much weaker than colchicine's. Dr. Wilson indicated that several thousand microtubules function in a structure known as the mitotic spindle in separation of chromosomes to daughter cells. Microtubules attach to the kinetochore of each chromatid and work together in chromosome movement. It appears that benomyl acts as colchicine does to form complexes with tubulin that then attach to microtubule assembly ends and effectively "cap" the polymer. If a sufficient number of microtubules are prevented from polymerizing chromatid separation will be blocked. He asserted that a dose response curve for this effect and a no inhibitory effect concentration can be determined. Dr. Wilson further stated that a concentration that does not inhibit mitosis completely, but that alters chromatid segregation leading to aneuploid cells might occur, but the dose range for this would, in his opinion, be very narrow and not be far below that needed to cause complete blockage. Therefore, Dr. Wilson stated that a risk estimate could be made for this effect.

The SAP based its opinion on a threshold for benomyl on the kinetics of the affinity for the same tubulin binding site of colchicine and benomyl, and the resulting inhibition of polymerization of tubulin to microtubules. The Panel's opinion was strengthened by studies with mammalian cells it cited (referenced in the SAP submission as Cox and Puck, 1969; Wilson et al., 1976) using the other well-known anti-mitotic agents, colcemid and the vinca alkaloids, as well as by a mouse micronucleus test with benomyl (referenced in the SAP submission as Seiler, 1977), all suggesting a threshold for spindle effects.

The Agency disagrees with the opinion expressed by the SAP, Dupont and Wilson (Mauer, 1980). A threshold for spindle effects and nondisjunction cannot be unequivocally demonstrated by the studies cited for either the antimitotic alkaloids, nor for benomyl. The *in vitro* studies (Cox and Puck, 1969; Wilson, Anderson, and Chen, 1976) were conducted with cell lines (CHO, EHB) displaying inconstant chromosome numbers; unsynchronized cell populations were treated; and no control cell clones were isolated for comparison to the treated isolates. The dose range between the concentration of agent needed to block movement of single chromosomes and that needed to cause complete mitotic arrest is extremely narrow (e.g., in the case of colcemid, 0.015 ug/ml and 0.03 ug/ml). Any reference to a mechanism involving what relative percentage of tubulin must be bound to account for selective inhibition of polymerization leading to "non-disjunction" is tenuous. The above data also show that only 3 percent of bound cellular tubulin appears to be critical to block the entire spindle thus leading to polyploidy. Cellular tubulin is involved as well in cellular processes other than the mitotic spindle. There is no evidence for thresholds involved in these processes.

The in vivo study cited (Seiler, 1977) was actually performed with methyl benzimidazole carbamate (MBC), the principle active metabolite of benomyl. The author reported no adverse effect (no increase in micronuclei) of MBC given orally to mice at a (single) dose of 50 mg/kg corresponding to a blood level of 8 ug/ml (the solubility limit of the compound). Further, the inference of a threshold for spindle effects (microtubular) was drawn by the investigator from his in vitro experiments with isolated pig brain preparations, in which 5×10^{-5} M (approximately 10 ug/ml) was the minimal effect level (25% inhibition of tubulin association), while 10^{-4} M (12 ug/ml) gave maximum effect (100% inhibition). Finally, the micronucleated erythrocytes which are counted as the end-effect in this test are not exclusively the product of spindle inhibition; micronuclei also occur following treatment with chromosome breakers. No effect levels one fifth to one tenth those for micronuclei have been reported with benomyl/MBC for reproductive effects in rats at 7.5 mg/kg, only a portion of which may be attributable to the chemical's effects on tubulin. Hence, the circulating threshold may conceivably be <8 ug/ml for these effects.

c. Teratogenic Effects

DuPont (2(30000/23B) stated that an ample margin of safety existed to protect the general public exposed to benomyl residues in treated crops from teratogenic effects. DuPont did not agree with the Agency's contention that a NOEL allowing for calculation of risk from dietary exposure did not exist. DuPont stated that a teratology study in which dietary administration was employed was entirely appropriate for calculation of an MOS. No teratogenicity was observed at doses as high as 5000 ppm in the diets of rats in DuPont tests. Using this NOEL, the teratogenic MOS for background dietary exposure would be about 20,000.

DuPont stated that the MOS for female mixer/loaders was ample. DuPont asserted that EPA used data of questionable validity in estimating exposure and ignored recent DuPont data obtained on benomyl itself. DuPont criticized EPA for not identifying the number of aerial mixer/loaders who are women of childbearing age for each use of benomyl. DuPont has not encountered women doing mixer/loader work. However, if it is assumed that there are women of child-bearing age at risk, the DuPont data indicate that the MOS is at least 400.

The SAP agreed that a NOEL for teratogenesis from benomyl administered by gavage to rats had not yet been established. Conclusions by the SAP favoring continued use of benomyl were contingent upon a NOEL for reproductive toxicology being established in studies then underway by DuPont and EPA. Furthermore, this level should provide a satisfactory MOS relative to dietary and worker exposure levels. SAP noted that dietary studies of the teratogenic properties of both benomyl and MBC in rats had been carried out and failed to show an effect at dose levels of 400 mg/kg/day. Teratogenic studies in rabbits at approximately 125 mg/kg/day in the diet also showed no evidence of compound related teratogenicity. Therefore, the SAP stated that any NOEL established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in dietary studies.

The Agency is aware that the results obtained by the two methods may differ. However, the Agency notes that both gavage and dietary administration have produced toxicologically significant results in animal tests (see positive oncogenicity results discussed in Section II.A. of this position document) (Sochard 1982).

The Agency, as discussed in Section II.F.3. of this document, considers gavage dosing to be more relevant than dietary dosing for extrapolating human risk from chemicals like benomyl which have short half-lives. Even so, the Agency considers that gavage may be a more conservative indicator of this risk and in interpreting MOS's, the Agency has taken this into consideration. The Agency estimates indicate margins of safety ranging from 86 to dietary background levels without a dust mask and from 600 to dietary background levels with a dust mask (Table 8) for non-dietary exposure. Margins of safety ranging from 254 to 600,000 were estimated for maximum single serving dietary doses of benomyl (Table 9). The Agency cannot affirm or refute DuPont's assertion regarding the existence of female mixer/loaders nor is it aware of data indicating what proportion of the benomyl mixer/loader population is female.

d. Exposure Estimate

USDA (5(30000/23B)) stated that theoretical calculations and actual data USDA (DuPont study indicating 0.016 mg/kg inhalation exposure) show a very adequate MOS with regard to reduction of spermatogenesis and birth defects. USDA suggested that calculations in EPA's PD 2/3 appear to be in error and should be recalculated. Further, USDA stated that it does not consider benomyl to constitute a hazard since it is non-volatile, it is rapidly metabolized in mammalian systems to non-toxic metabolites, there is no exposure by the dermal route, dietary intake is not of concern (per EPA PD 2/3 conclusion), and that use of a cloth mask would significantly reduce the inhalation exposure.

DuPont stated (2(30000/23B)) that an ample MOS to protect workers from reduction in spermatogenesis exists. Using extensive recent field monitoring exposure data obtained with commercial aerial mixer/loaders actually loading benomyl, the average MOS was at least 400. The EPA calculation of an MOS of 21 for aerial mixer/loaders used data from Jegier (1964) to estimate mixer/loader inhalation exposure. No reference to the DuPont mixer/loader study was contained in the PD 2/3. The DuPont study was submitted in April, 1979; other information supplied to EPA at that time was incorporated into the EPA risk assessment. No information was provided in the PD 2/3 to compare particle size or dustiness of the product used by Jegier with that of benomyl. The Jegier study used only three subjects; DuPont's involved ten subjects. Jegier's article did not indicate how much product was actually loaded. DuPont stated that exposure will be a direct function of the number of pounds handled, and since Jegier's data were expressed in terms of time worked, no basis for comparison of these data existed.

DuPont stated that EPA's calculation using estimates provided by USDA for the number of hours worked per day was inappropriate. The critical factor in calculating exposure is not the hours on the job, but actual pounds loaded. DuPont estimated exposure based upon assumptions about how much Benlate® a mixer/loader would handle in one day. They stated that average inhalation

exposure loading an average of 42 pounds was 0.08 mg benomyl. The average pounds loaded per day was 500 giving an average inhalation exposure of 0.95 mg (500 divided by 42). The daily dose for a 60 kg person would be 0.016 mg/kg. Assuming a testicular NOEL in male rats of 7.5 mg/kg/day the MOS would be 468 (7.5 divided by 0.016).

Mr. W.J. Walla (4(30000/23B)) offered the following comments on exposure:

"Jegier et al. (1964) made the following comment regarding applicator exposure:

'The overall results indicate the hazard associated with spraying of crops is a very low order due to low or limited periods of exposure.'

Wolfe et al. (1967) commented on the potential hazard of applicator exposure:

'Observations have indicated that the number of hours per day or per week the worker is actually loading airplanes is quite low. Secondly, in such a situation where it is obvious that high contamination of the worker may occur, much more attention is generally given to the use of adequate protective clothing and respiratory devices than in less hazardous jobs. Thirdly, probably only a small percentage of the dry dust impinging on exposed skin areas is actually absorbed.'

Dr. Walla further stated that to determine actual mixer/loader exposure or potential for exposure, data developed by the USDA EPA Benomyl Assessment Team should be examined. The two uses in question are on rice and soybeans as applied by aerial applicators. About 300,000 pounds of benomyl were applied to rice by 80 aerial applicators. There are 200 mixer/loaders involved in the loading operation or 2.5 mixer/loaders per applicator. The first application to rice is made about June 12 and the final application on July 20 for a total spray period of 38 days exposure, then each applicator would spray 3,750 pounds of benomyl (300,000 divided by 80) or approximately 187.5 pounds of benomyl per day (3,750 divided by 20). This means that each aircraft would apply 375 pounds of Benlate® per day and each mixer/loader would be exposed to a maximum of 150 pounds of Benlate® per day for a total of 20 days per year.

About 760,000 pounds of benomyl was applied by 200 aerial applicators with an average of 2.5 mixer/loaders per applicators to soybeans. The first applications are made on July 31 and the final applications on September 22 or a total of 52 days. If the actual spraying took place over a 20 day period then each applicator would apply 3,800 pounds of benomyl during the season (760,000 divided by 200) or 190 pounds per applicator per day or 152 pounds of Benlate® per mixer/loader per day (380 divided by 2.5). These figures are considerably less than the exposure calculated by the EPA from the same source.

Dr. Walla, as Chairman of the Assessment team, felt that these exposure levels were significantly low enough that the use of a dust mask would virtually

eliminate all exposure hazards and increase the factor of safety to the point that requirements for water soluble packaging and an extra warning on the label were unwarranted.

When the Agency performed the exposure assessment for benomyl, actual data on exposure to mixer/loaders were not available. Therefore, the Agency performed the assessment with data from a study using a 25% Guthion® WP (Jegier, 1964). The average inhalation exposure per hour calculated using the Jegier data was 2.5 mg/hour; the average inhalation per hour calculated using DuPont's data (Eberhart and Holt, 1982) was 1.2 mg/hr with ranges from 0.06 mg/hr to 4.5 mg/hr. The Jegier data were within the range observed in the DuPont study, therefore, the two data bases were comparable (Saito, 1982d).

The Agency used the DuPont study to evaluate the claim that no basis existed for comparing data expressed in terms of time worked with data based on pounds handled. That study indicated that about 15 pounds of Benlate® can be mixed per minute. If it is assumed that 500 pounds are loaded per day, the time required for actual mixing would be about 34 minutes. The daily dose based on time needed to load 500 pounds would then be

$$\frac{(1.2 \text{ mg/hr}) \times (0.56 \text{ hr/day})}{70 \text{ kg/person}} = 0.01 \text{ mg/kg/day.}$$

The daily dose based on pounds loaded would be

$$\frac{0.03 \text{ mg/42 pounds} \times 500 \text{ pounds/day}}{70 \text{ kg/person}} = 0.014 \text{ mg/kg/day.}$$

Therefore, the exposure is comparable whether it is determined by poundage or time. Data supplied to the Agency by the USDA was reported on the basis of hours worked, not in pounds used for most of the crops. The estimate of the time spent in mixing/loading was very imprecise. Information on pounds loaded per day was not supplied (Saito, 1982d).

e. Spermatogenic Effects

DuPont further stated that EPA's calculation of the spermatogenic effects MDS from dietary exposure (380) was apparently obtained by dividing the estimated maximum dietary exposure 0.02 mg/kg/day into the inhalation NOEL for testicular effects (7.5 mg/kg day). There is no scientific basis for such a calculation. To estimate risk from dietary exposure there is no need to, or basis for extrapolating from inhalation data. Results from animal feeding studies are available and should be used. In a two-year feeding study in rats, no testicular effects were observed at 2,500 ppm (125 mg/kg) in the diet. No compound related testicular effects were seen in a 2-year dog feeding study at 2,500 ppm (62.5 mg/kg). This provides an MDS of about 5,000, not 380 as stated in the PD 2/3.

The Agency has evaluated the above studies cited by duPont and other studies in arriving at its determination on benomyl. Inhalation studies of compounds like benomyl provide input in evaluating dietary effects because of the common physiological response of swallowing some of the materials that become

dissolved in nasopharyngeal secretions when inhaled. Furthermore, data from Ravlock, et al. (1982) show reduction in testes of mature pups from benomyl-dosed (gavage) dams treated during gestation and lactation (Sochard, 1982).

2. Thiophanate-Methyl

a. Gene Mutation Tests

The SAP concurred with the Agency's position to terminate the RPAR against thiophanate-methyl. In regard to the option of requiring additional gene mutation tests for MBC, a metabolite of this compound, the SAP offered the same comments as discussed in Section IV.A.1.a. above for benomyl with respect to an appropriate test battery.

In evaluating the data available on the mutagenicity of MBC, the SAP also looked at mutagenicity studies with benomyl since studies have shown that at least 80 percent of benomyl is metabolized to MBC *in vivo*. On examination of the mutagenicity tests for MBC, benomyl and thiophanate-methyl, the SAP stated that the requirement for a sophisticated microbiological test had been met. In addition, the SAP stated that a suitable *in vivo* cytogenetics assay had been completed. However, the SAP did not believe that an appropriate mammalian cell point-mutation assay had been performed and, therefore, it recommended that such an assay be conducted using MBC and a mouse lymphoma or Chinese hamster embryo cell line or other equally well validated test for point mutations in mammalian cells. The SAP indicated that the mutagenic potential of thiophanate-methyl should be considered to have been completely rebutted and no further tests should be required if the results of this test were negative.

The Agency has received no additional data on the mutagenic potential of thiophanate-methyl, therefore, the issue of its potential as a point mutagen is not resolved.

B. Comments Relating to Benefits

DuPont (2(30000/23B)) stated that the Agency's benefits analysis relied solely upon the report of the USDA/State/EPA Benomyl Assessment team and concluded that the benomyl rice use provided a net economic benefit of about \$15 million.

DuPont submitted a report entitled, "Economic Benefits of Benomyl" prepared by Arthur D. Little, Inc. that estimated the annual loss to rice growers if benomyl were not available to be \$22 million; the annual loss to the total national economy as a result of the rice use loss would be \$73 million.

DuPont stated that plant diseases caused by fungi result in an estimated \$5 billion annual loss in the United States despite current control measures. Most crops suffer from attacks of different species of fungi. Fungal diseases are sporadic in occurrence and may cause only a few percent loss in yield one year and a complete crop loss the following year. In general, fruit and vegetable crops suffer more heavily than field crops; losses from disease occur both in the field and postharvest, while in process, transit or storage. In

the A. D. Little study the national economic benefits of benomyl usage versus available alternatives were estimated at over \$4 billion during the time period 1978-1982 (assuming that all other fungicides would still be available and in plentiful supply for all currently registered uses).

Dupont concluded that benomyl is unmistakably a unique compound much needed and widely used, not only in American Agriculture's struggle to protect crops from attack by fungal disease, but in other agricultural areas of the world as well.

The Agency agrees that the overall benefits of benomyl use are large since the estimated economic impacts upon pesticide users exceeded a range of \$26 to \$52 million for the limited number of sites that were analyzed. These sites accounted for 0.81 to 1.03 million pounds active ingredient, which was approximately one-third of the total U.S. benomyl market.

Since the Agency did not assess all benomyl use sites in its economic impact analysis, considerable divergence exists between the Agency's estimates and those submitted by DuPont. Given biological and economic data limitations, the Agency's approach was mostly limited to qualitative assessments at the producer level; economic assessments of impacts upon final consumers or other interdependent sectors of the US economy would be less than definitive and somewhat tenuous.

In addition, the Agency did not aggregate economic impacts over a succession of years, but presented economic impacts in terms of a typical year immediately following a hypothetical cancellation. Inherent biological and economic data limitations, as well as future market uncertainties for both factors of production (e.g. the development of new fungicides) or agricultural outputs, would be expected to distort longer term estimates. Without proper consideration given to the uncertainty of these longer term estimates, faulty analytical conclusions may result.

Though the analytical approach of the Agency and DuPont are different, both analyses indicate that the economic impacts upon benomyl users would be significantly large if this pesticide were no longer available for use. The Agency also recognizes that there would be economic impacts upon final consumers, as well as other interdependent sectors of the US economy, and that these impacts would be expected to occur over some extended period of time. However, the Agency contends that existing data limitations and the uncertainty of markets are deterrents to highly accurate forecasts of these impacts (Gaede, 1982).

C. Comments Relating to the Regulatory Decision

1. Respiratory Protection

The SAP supported the requirement that a protective mask be worn during the mixing, loading and transfer operations for aerial application of benomyl. However, the SAP recommended the use of commercial disposable paper masks be allowed if, in the opinion of the Agency, they provide sufficient protection against exposure to benomyl during these operations.

The USDA (5(30000/23B)) also concurred with the Agency decision to continue registration of benomyl and agreed that a fine woven cloth mask requirement would reduce exposure of aerial mixer/loaders to benomyl.

DuPont stated (2(30000/23B)) that there is an ample margin of safety to protect the aerial mixer/loader from toxic effects that could result from inhalation exposure. Thus, no mask is needed; however, should the Agency decide that some additional protection is desirable for this group of workers, DuPont agrees that a mask is a more appropriate regulatory option than water-soluble packaging.

The Agency agrees with the SA³ that the use of a commercial disposable paper mask would provide sufficient protection (Saito, 1982e). Therefore, the requirement for a dust mask can be satisfied by use of either a finely woven cloth mask or disposable paper mask.

2. Label Changes

The SAP did not concur in the suggested precautionary warning statement to be included on the label for benomyl. The basis for their non-concurrence was that if this type of labelling would be required for benomyl it should also be required for all other pesticide products which cause adverse health effects in man. The SAP might favorably consider specific health effects warning statements on pesticide labels were this requirement uniformly applied to all pesticide products. However, this selective requirement for a warning statement on benomyl was not appropriate in view of past regulatory decisions that have been made relative to other pesticide products that have undergone the RPAR process.

USDA (5(30000/23B)) urged the Agency to reconsider the proposed label statement regarding reduced sperm count and birth defects in laboratory animals. USDA felt that warning statements should be reserved for demonstrated risk situations. USDA did not consider benomyl to present a hazard and, therefore, such a statement would mitigate the effectiveness and credibility of subsequent warnings.

Dr. W. J. Walla (4(30000/23B)) indicated that "In my opinion label requirements have already been established for pesticides with 'Caution', 'Warning', and 'Danger' signals. We in the field have been educating users on the meaning of these signal words and additional warnings on the label would confuse the issue. Further, the EPA would be setting a precedent for future RPAR'd compounds that might be difficult to continue especially with the more toxic compounds."

DuPont (2(30000/23B)) objected to the label statement on the basis of the following: Present label warnings are sufficient, an ample MOS exists to protect against these risks, such a statement would diminish the impact of more critical hazard warnings, the proposed statement is excessive, label statements imposed haphazardly impart an unfair competitive disadvantage.



The Bonide Chemical Company (1(30000/23B)) commented that the Agency's proposed label warning for five-pound or larger bags should apply only to products of greater than or equal to 50 percent active ingredient. Their 1.6 percent lawn care product for homeowners should be exempt from additional warning statements. The statements would confuse the homeowner and they are unnecessary since they do not use the product every day and, therefore, do not sustain levels of exposure estimated by the Agency in the PD 2/3.

The Agency has withdrawn the proposal for additional warning labelling for benomyl since the risk analysis contained herein indicates that risks from most exposure situations are low when compared to the benefits and for the highest risk situation, mixer/loaders for aerial application, they became low when a dust mask is used while engaged in the activity. Therefore, such labelling is no longer considered necessary to control the risk.

3. Monitoring Studies

The SAP supported the requirement for additional monitoring studies on benomyl for the rice use to allow the Agency to make a more accurate determination of the potential risks to aquatic organisms from this use.

DuPont (2(30000/23B)) stated that there is already ample information available to conclude that there is no significant risk to aquatic organisms from the use of benomyl on rice. Nevertheless, they indicated that they would be willing to cooperate in conducting an additional study.

The Agency has reviewed the information on this issue and concluded that there is insufficient evidence to conclude that there is no risk attributable to the rice use. Uncertainties that remain concerning the extent of aquatic contamination underline the need for a comprehensive monitoring study in order to resolve this issue (Stevens, 1982). The Agency and DuPont will cooperate to design an appropriate protocol for this study (see further comments on monitoring under Section IV.D.).

4. Water-Soluble Packaging

The SAP indicated that water-soluble packaging should not be required since the Agency was requiring use of a cloth mask to reduce exposure to aerial application mixer/loaders.

The USDA (5(30000/23B)) also had reservations regarding the water-soluble packaging requirement for five pound or larger quantities. They stated that protection of water soluble bags from the elements might be required. Benomyl stored in such bags might sustain losses or cause human or environmental hazards if they were exposed to high humidity, dew, rain or damp storage. The five pound bag size limitation might create disposal and/or overapplication problems when quantities other than five-pound increments must be used.

DuPont (2(30000/23B)) stated that water-soluble packaging is not needed since there is already an ample margin of safety to protect workers. Further EPA's cost estimates were low for this packaging (out of date). DuPont also stated that such packaging is not accepted by all growers.

Since commenting on this requirement, DuPont has provided information to the Agency relative to use of water-soluble films for packaging of benomyl in Florida (Julin, 1981). They concluded that such packaging was impractical and uneconomical. Problems developed with maintaining the product's guaranteed strength in this situation.

The Agency agrees with the commentators that both water-soluble packaging and a mask need not be used at the same time. The Agency is aware that technical problems must be overcome before water-soluble bags can be used. DuPont has encountered problems with benomyl reacting with the film. Therefore, the Agency agrees that water-soluble packaging for benomyl is not technically feasible at this time.

The Agency disagrees with USDA that greater care would be needed in protecting benomyl packaged in water-soluble bags than is presently required in protecting benomyl from the elements. The current DuPont benomyl label cautions against permitting "Benlate®" to become wet during storage. The Agency did not suggest that the water-soluble bags should contain five pounds of benomyl. Pesticides currently sold in water-soluble bags (including experimental sales of benomyl) are usually packaged in one-half pound bags (i.e. ten bags per five pounds).

D. Comments Relating to Aquatic Hazard

Dr. Walla, (4(30000/23B)) provided information on the aquatic hazard issue. He indicated that water released from a rice field is usually drained into a field ditch where it flows to a secondary canal. From the secondary canal it could be diverted to a second field being reflooded for second crop rice production or be emptied into a main canal. Once in the main canal it could flow into a main waterway. Catfish farmers do not normally use water drained from a rice field for several reasons. Rice fields are drained during July and August, when water temperatures are approaching 100 degrees F and the oxygen supply in the water is extremely low. The high water temperature and low oxygen supply would kill fish in the absence of toxic substances. When fish are farmed commercially, diseases and trash fish are serious problems and catfish farmers use well or spring water as a water source to eliminate these problems. It would be highly unlikely that a commercial fish producer would use water released from rice fields for fish ponds.

In addition, DuPont (2(30000/23B)) stated that when benomyl is used according to label directions, there is no significant risk to aquatic organisms because of the low level of exposure. DuPont contended that benomyl was not registered for any use involving its direct application to water. Furthermore, aquatic organisms are not exposed after the application of benomyl to rice, since fish are not grown in rice fields. DuPont agreed that there is water in rice fields at the time of application, but benomyl is not applied directly to that water. Some very small residues from treated rice fields are drained into natural bodies of water. However, DuPont maintained that those residues that leave rice fields are insignificant biologically and have no adverse effect on aquatic organisms because of dilution.

DuPont further stated that EPA's PD 2/3 acknowledged that the only evidence of fish kills had resulted from misuse. In passing the Federal Pesticide Act of 1978, Congress specifically stated that interim administrative review of a pesticide should be based only on validated tests or other significant evidence; and that it was their intent that other "significant evidence of adverse risk means factually significant information and is not to include evidence based only on misuse of the pesticide." DuPont stated that the courts also firmly established that adverse effects resulting from misuse could not be the basis for regulatory actions.

DuPont does not agree with the Agency claim that it was justified in citing two cases of misuse, because the Agency contended that draining rice fields into catfish ponds was a common practice. DuPont stated that it is not a common practice to drain rice fields into catfish ponds. DuPont provided literature to the Agency in a supplemental response dated June 6, 1978, showing that catfish growers must use extreme care in obtaining water. USDA recommends that water used in catfish farming should be drawn from wells; surface water is generally unsuitable for many reasons, including its possible contamination with toxic pesticides other than benomyl.

DuPont contended that the Agency could quantify risk based upon the study of rice field soil and water residues submitted in its 1978 RPAR rebuttal. The highest residue found in rice field water was 90 ppb. Although this exceeded 1/2 the acute LC_{50} of channel catfish, catfish are not found in rice fields. Further, DuPont stated that a total of 30 water samples were taken outside of the rice fields at the time of draining. Traces of benomyl were found in some drainage ditches. In only one canal sample was a detectable level (40 ppb) of benomyl found. No other detectable residues were found in canals, streams or lakes even a short distance away. Soil and water chemistry data provided to the Agency are consistent with, and explanatory of, these observations. Regardless of theoretical calculations and attempts to measure residue levels in soil and water, the absence of fish kills in actual use is the most compelling evidence that there is no significant risk to aquatic organisms from the use of benomyl on rice.

The Agency concluded in its PD 2/3 that benomyl's use on rice posed a significant risk to natural local populations of catfish, other Ictalurids, and fish food organisms. The Agency is still concerned with the exposure of non-target organisms in bodies of water that may contain residues of benomyl or its degradation products after application to rice. This hazard is significant because rice fields are in close proximity to surface waters. Current agricultural practices include flooding and subsequent drawdown and/or drainage of fields. The extent of aquatic contamination of aquatic sites is still uncertain. DuPont's study submitted in its 1978 RPAR Rebuttal was insufficient evidence to conclude that there is no risk attributable to this use. (Stevens, 1982).

Further, the lack of reported wildlife incidents is often alleged to support product safety claims. As previously stated, the only evidence of fish kills so far has resulted from misuse of benomyl. However, this may be more related to monitoring efforts expended to detect such incidents and the lack of

proximity and accessibility of such sites to human activity. Reported incidents may be a small sample of regularly occurring phenomena and are therefore not substantiation of no wildlife effects from a compound.

The Agency agrees that problems in commercial catfish farms due to benomyl contamination of water are not the main concern. The use of rice field drainage water in such fish ponds may or may not be a reasonable common practice and no attempt is being made to regulate benomyl in this regard past the warning statements already on benomyl product labels.

V. Conclusions and Requirements

A. Risks

The Agency has made the following conclusions on the oncogenic, mutagenic, teratogenic, spermatogenic and aquatic risks posed by benomyl and the oncogenic risks posed by thiophanate-methyl:

Both benomyl and MBC, the common metabolite of both benomyl and thiophanate-methyl, have been shown to be hepatocarcinogens in tests with mice. Based upon the most sensitive parameter for this effect, liver tumors from the DuPont study with MBC, the upper limit to the oncogenic risk to the general public via worst case dietary exposure was estimated to be 7×10^{-5} if food residues exist at tolerance levels and if 100 percent of crops with benomyl and thiophanate-methyl registrations are treated. The upper bound of the dietary risk estimate when the percent of crop treated is considered, becomes about 3×10^{-5} . If average measured residues are considered, the upper bound of dietary oncogenic risk would be about 7×10^{-6} . For workers/users the upper bound of the oncogenic risk without respiratory protection ranges from 5×10^{-7} to 3×10^{-5} and if a dust mask is used from 7×10^{-7} to 5×10^{-6} . The oncogenic risks presented by thiophanate-methyl are assumed to be similar and not greater than those presented by benomyl since both fungicides are similar in use and metabolism.

The worker exposure estimates used in generating the oncogenic risk estimate assume that all of the inhaled product is absorbed from lung tissues and that this material is completely converted to MBC after being absorbed. In addition, maximum estimates of hours involved in work related activities were used since precise data were not available. These factors tend to maximize the risk estimate. Evidence exists which suggests that both the work related exposure and dietary exposure could be an order of magnitude lower than worst case calculations. Therefore, the Agency assessment of the potential oncogenic risks associated with benomyl and thiophanate-methyl, does not raise prudent concerns for unreasonable risk when compared to the benefits of use of these fungicides. The Agency is not initiating an RPAR against these products based upon the oncogenicity risk criterion.

Benomyl and the MCB metabolite of benomyl and thiophanate-methyl have been shown to cause effects to the cellular spindle apparatus. The impact of this effect to human health cannot be adequately assessed at this time. Therefore, mutagenic risk in the form of heritable spindle effects or point mutagenicity do not now lead to a recommendation for regulatory action. However, the data on mutagenicity are supportive of the qualitative determination of benomyl's potential teratogenic, spermatogenic and oncogenic effects and thiophanate-methyl's potential for oncogenic effects.

Benomyl has the potential to cause teratogenic effects. The NOEL for these effects is tentatively set at 30 mg/kg/day based on results from a gavage study in rats. DuPont is now testing to confirm this level. A number of dietary studies of teratogenicity have reported negative results. For worker/users MOS's range from 86 to dietary background levels without respiratory protection and from 600 to dietary background levels if a dust mask.

is used. Margins of Safety (MOS) for teratogenicity from dietary exposure range from 254 to 600,00 (single serving basis). The Agency notes that these MOS's for dietary risk are derived from a NOEL determined in a gavage teratology study. In general, the Agency considers the gavage route of dosing to be the method of choice for determinations of the NOEL for such effects. Further, for compounds such as benomyl, which are minimally absorbed and rapidly excreted from the system when taken in the diet, the Agency considers that risk extrapolation from gavage studies is appropriate because it more closely approximates human dietary exposures which peak periodically with ingestion of meals. Even so, the Agency recognizes that MOS's so derived for such chemicals are conservative risk indicators and this is considered in the Agency's evaluation of teratogenicity.

Benomyl's potential to cause spermatogenic effects is shown by tests using various dosing routes. The NOEL for these effects is 7.5 mg/kg/day based on an inhalation study in rats. MOS's for spermatogenic effects from dietary exposure range from 64 to 150,000 (single serving basis). MOS's for workers/users for aerial application range from 21 to dietary background levels without respiratory protection and from 150 to dietary background levels if a dust mask is used.

The Agency is not able to determine the level of risk to aquatic organisms from the use of benomyl in rice fields at this time. Additional field monitoring for benomyl residue levels in water will be required by the Agency.

B. Benefits

Benomyl is one of the more important fungicides for use on fruit crops. The Agency's brief review of benomyl's benefits for selected crops indicates that economic impacts from the inavailability of benomyl would range from greater than \$26 million to greater than \$52 million in a typical year for treatment costs alone. Additional impacts for lost production and higher consumer prices would also be expected, however, the Agency was unable to estimate the magnitude of these additional losses.

The benefits of thiophanate-methyl have not been quantified. This fungicide is currently undergoing an expansion of its use registrations with most new uses being established for less than two years. If equivalent efficacy with benomyl is assumed based upon both product's fungitoxic action being the result of their common metabolite, MBC, then the current level of thiophanate-methyl benefits would have the potential for increasing to a degree dependant upon its ability to become market competitive with benomyl.

In aggregate, the joint benefits of thiophanate-methyl and benomyl are large. These could increase if increased market competition between both products leads to price reductions since this would be expected to increase the overall usage of the products.

C. Action

The Agency has determined that the potential oncogenic, teratogenic, and spermatogenic, risks of benomyl and the oncogenic risk of thiophanate-methyl are exceeded by the benefits associated with the use of products containing these chemicals, therefore, the Agency is concluding the RPAR action for both benomyl and thiophanate-methyl. The Agency has decided to implement the proposed action as set forth in the Benomyl Position Document 2/3 (US EPA, 1979c) with a few modifications. A comparison of the regulatory action proposed earlier and this decision is presented in Table 11. The regulatory action which the Agency will implement is discussed in this section.

The Agency has determined that the use of a dust mask while mixing and loading benomyl products could decrease inhalation exposure by as much as 90 percent. The Agency has determined that the teratogenic and spermatogenic risks associated with mixing and loading of benomyl products for aerial application would be exceeded by the benefits of benomyl use if a dust mask is worn during this activity. Therefore, the Agency will require the use of a cloth or disposable paper mask for respiratory protection of workers engaged in mixing and loading of benomyl for aerial application. A statement to this effect shall be added to benomyl-containing products in five pound or larger packages which are labelled in a manner which permits aerial application.

Since data do not exist that permit the Agency to determine the level of aquatic risk associated with the use of benomyl on rice, benomyl registrants will be required to perform a test to determine the level of benomyl residues in various aquatic sites near rice fields. Details of testing protocol will be determined in meetings between the registrants and the Agency.

The Agency will not require the use of water soluble packaging for benomyl products intended for aerial application at this time. The requirement is not considered necessary at this time since use of a mask by workers during mixing and loading of benomyl for aerial application will decrease benomyl exposure to acceptable levels.

The Agency will not require a label warning for birth defects and reduction of sperm levels at this time. This statement, as proposed in PD 2/3, is no longer considered necessary in view of the risk reduction achieved by use of dust masks during mixing and loading of benomyl for aerial application.

The Agency will not require additional mutagenicity testing for benomyl or thiophanate-methyl products at this time. Additional information on benomyl was received subsequent to publication of the PD 2/3.

Table 11
Comparison of Proposed Regulatory Actions

Proposal	PD 2/3	PD 4
<u>Benomyl</u>		
1. Addition of a warning statement regarding birth defects and reduced sperm production to products in five pound or larger packages.	Required	Not Required
2. Require water-soluble packaging for benomyl products sold in five pound or larger sizes.	Required	Not Required
3. Require additional gene mutation studies.	Required	Not Required ¹
4. Require use of a cloth mask for workers during mixing and loading of benomyl for aerial application.	Required	Required ²
5. Require performance of a monitoring study of benomyl residues in water rice fields treated with this fungicide.	Required	Required

- ^{1/} Studies were submitted by DuPont since publication of the PD 2/3.
^{2/} A commercially disposable paper mask is also acceptable.



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APPENDIX 1
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

November 30, 1979

OFFICE OF TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of FIFRA Section 6(b)(1) Action on Benomyl and Thiophanate-methyl

FROM: Dr. H. Wade Fowler, Jr. *H. Wade Fowler, Jr.*
Executive Secretary
FIFRA Scientific Advisory Panel (TS-766)

TO: Deputy Assistant Administrator
for Pesticide Products (TS-766)

The FIFRA Scientific Advisory Panel has completed review of the Notices of Determination concluding the Rebuttable Presumptions Against Registration (RPAR's) of pesticide products containing benomyl and thiophanate-methyl. The review was completed during open meetings held in Arlington, Virginia, during the periods October 9-10, 1979, and November 29, 1979.

Attachment

cc: Panel Members
Mr. Conlon
Esther Saito
OPP Division Directors

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA)

SCIENTIFIC ADVISORY PANEL

Review of Preliminary Notices to
conclude Rebuttable Presumptions Against Registration
(RPARS) of products containing benomyl and thiophanate-methyl

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel has completed review of plans by the Environmental Protection Agency for initiation of regulatory action on benomyl and thiophanate-methyl pesticide products under the Provisions of Section 6(b)(1) of FIFRA, as amended. The review was completed during open meetings in Arlington, Virginia, during the periods October 9-10, 1979, and November 29, 1979.

Maximum public participation was encouraged for the review. Federal Register Notices were published on September 21, 1979, November 14, 1979, and November 27, 1979. In addition telephonic calls and special mailings were sent to the general public who had previously expressed an interest in activities of the Panel.

Written and oral statements were received from representatives of E. I. DuPont Nemours and Company and Pennwalt Corporation in respect to proposed regulatory action on benomyl and thiophanate-methyl, respectively.

In consideration of all matters brought out during the meeting and careful review of all documents submitted by the Agency and other parties, the Panel unanimously submits the following report:

Panel Position on Thiophanate-methyl

The Scientific Advisory Panel concurs with the Agency's position to terminate the RPAR against thiophanate-methyl.

In regard to the option under consideration by the Agency for additional gene mutation tests for MBC, a metabolite of thiophanate-methyl, the Scientific Advisory Panel offers the following comment:

In May 1978, the FIFRA Scientific Advisory Panel issued the following statement relative to the mutagenesis subsection of subpart F, Hazard Evaluation: Human and Domestic Animals, of the Proposed Guidelines for Registering Pesticides in the United States:

"The Scientific Advisory Panel strongly endorses the need for mutagenic assays as a survey for potential environmental hazards and endorses the principles of the proposed guidelines. Moreover we feel that mutagenesis and oncogenesis should be considered together as related rather than isolated phenomena. To these ends, therefore, and in the interest of simplicity and practicality, the following core battery of tests selected from the proposed guidelines is recommended for mutagenicity screening of all pesticides.

1. A sophisticated microbiological test such as an enhanced Ames assay with appropriate dose response.
2. A mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma.
3. An in vivo cytogenetics assay.

In addition the Panel recommends that a multiple generation test which is part of the reproductive study (162.83-4) be modified to provide a statistically significant assay for dominant lethal mutations. The Panel also recommends that oncogenicity studies (162.83-2) be used in the overall evaluation of mutagenicity. Finally the Panel recommends that both the core battery of tests outlined in this report and the dominant lethal test be performed on each pesticide. The results of these tests will provide the Agency with the information necessary to estimate the mutagenic potential of pesticides".

In evaluating the data available on the mutagenicity of MBC, the Scientific Advisory Panel has also evaluated mutagenicity studies with benomyl since metabolism studies have shown that at least 80% of benomyl is metabolized to MBC in vivo. On examination of the mutagenicity tests that have been conducted using MBC, benomyl, and thiophanate-methyl, the Panel is of the opinion that the requirement

for a sophisticated microbiological test has been met. In addition, the Panel is of the opinion that a suitable in vivo cytogenetics assay has been completed. However, the Panel does not believe that an appropriate mammalian cell point-mutation assay has been performed. Therefore, we recommend that a mammalian cell point mutation assay be conducted using MBC and a mouse lymphoma or Chinese hamster embryo cell line or any other equally well validated test for point mutations in mammalian cells. If the results of this test are negative, the Scientific Advisory Panel is of the opinion that the mutagenic potential of thiophanate-methyl be considered to have been completely rebutted and no further tests should be required.

In regard to the potential for spindle effects from human exposure to thiophanate-methyl, the Scientific Advisory Panel offers the following conclusions: A negative result has been obtained in a multigeneration reproductive study and a dominant lethal study using thiophanate-methyl. The Scientific Advisory Panel is of the opinion that these two tests and an appropriate in vivo cytogenetics test constitute an adequate measure for adverse health effects in man from spindle poisons. Having met the proposed requirements the Scientific Advisory Panel is of the opinion that there is no significant risk from spindle inhibition in man as a result of exposure to thiophanate-methyl in its normal use in agriculture.

PANEL POSITION ON BENOMYL

In regard to the specific issues and questions posed by the Agency to the Panel regarding the benomyl RPAR, the Scientific Advisory Panel offers the following responses:

Questions from EPA Concerning Its Regulatory Decision.

Question 1. Based on the data available at this time do you agree with the agency's conclusion of low risk from the spindle effects associated with use of benomyl?

Answer: Yes (see Appendix I).

Question 2. Would you suggest a method of estimating the mutagenic risk for spindle effects?

Answer: See Appendix I.

Question 3. We proposed to request gene mutation tests in (1) *Drosophila*, (2) mammalian somatic cells in culture, and (3) an appropriate eukaryotic microorganism. In your opinion are these tests adequate?

Answer: See Appendix 1.

Question 4. During the April 1979 Scientific Advisory Panel meeting we discussed the DuPont dermal absorption study. Would you comment on this study and the agency's conclusion that the dermal component of the total body dose is extremely low.

Answer: We have examined the data from the study conducted by DuPont on dermal absorption of 2-¹⁴C-benomyl through rat skin. We concur with the agency's position that it would not be possible to obtain a level of benomyl in the blood through dermal absorption which would pose a significant risk.

Panel Comments on Benomyl Regulatory Decision

The EPA has proposed the continued registration of benomyl for all uses, but with an amendment of the terms and conditions of registration.

These amendments of terms and conditions of registration entail the requirement for (1) additional mutation studies, (2) the requirement for water soluble packaging for benomyl sold in units of 5 pounds or larger, (3) that a cloth mask be required during mixing and loading for aerial application if the product is not packaged in water soluble bags, (4) that a monitoring study of the fate of benomyl in the environment be required for continued registration for rice uses and, (5) that label changes will be required including a precautionary statement relative to reproductive hazards.

Mutation Studies (1)

Concerning the requirement for additional mutation studies see Appendix 1.

Water Soluble Packaging and Respiratory Protection (2) and (3)

The FIFRA Scientific Advisory Panel supports the requirement that a protective mask be worn during the mixing, loading and transfer operations for aerial application of benomyl. However, the Panel recommends the use of commercial disposable paper masks, in addition to the cloth mask specified in the decision document be allowed if, in the opinion of the agency, they provide sufficient protection against respiratory exposure to benomyl during these operations. With the recommendation for use of respiratory protection in mixing, loading and transfer operations involving benomyl, the Scientific Advisory Panel believes that the requirement for water soluble packaging for units of 5 pounds or larger should not be required.

Monitoring Studies (4)

The Panel supports the requirement for additional monitoring studies on benomyl for rice use. These studies will allow the EPA to make a more accurate determination of the potential risks to aquatic organisms of the use of benomyl on rice.

Label Changes (5)

The Scientific Advisory Panel does not concur in the suggested precautionary warning statement to be included on the label for benomyl. The basis for the Panel's non-concurrence is that if this type of labeling is required for benomyl it should also be required for all other pesticide products which cause adverse health effects in man. This, of course, would include almost all pesticide products currently in use. The Panel might favorably consider specific health effects warning statements on pesticide labels were this requirement uniformly applied to all pesticide products. However, we are of the opinion that the selective requirement for this kind of warning label on benomyl is not appropriate in view of past regulatory decisions that have been made relative to other pesticide products that have undergone an examination through the RPAR process.

Additional Comments

The no effect level for teratogenesis from benomyl administered by gavage to rats has not yet been established. Two studies, one by the Environmental Protection Agency and another by DuPont are currently underway in which the Scientific Advisory Panel has an interest. Any conclusions by the Panel favoring continued use of benomyl are contingent upon a no effect level for reproductive toxicology being established in these studies. Furthermore, the level at which no effect is seen should provide a satisfactory margin of safety relative to the exposure levels which have been calculated from dietary intake and exposure during mixing, loading, transfer or application of benomyl. It should be noted that dietary studies of the teratogenic properties of both benomyl and the breakdown product MBC in rats have been carried out and the results of these studies fail to show an effect at dose levels of 400 mg/kg/day. Teratogenic studies in rabbits at approximately 125 mg/kg/day in the diet also showed no evidence of compound related teratogenicity. Therefore, it is the opinion of the Scientific Advisory Panel that any no effect level established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in these dietary studies.

Except as noted above, the Panel concurs with the agency's position for continued registration of benomyl for all uses.

APPENDIX I

Mutation Studies

The Scientific Advisory Panel offers the following comment in regard to the agency's request that additional gene mutation tests using benomyl be carried out in Drosophila, mammalian somatic cells in culture, and in an appropriate eukaryotic microorganism.

On May 31, 1978, the FIFRA Scientific Advisory Panel issued the following statement relative to the mutagenesis subsection of subpart F, Hazard Evaluation: Human and Domestic Animals, of the Proposed Guidelines for Registering Pesticides in the United States:

"The Scientific Advisory Panel strongly endorses the need for mutagenic assays as a survey for potential environmental hazards and endorses the principles of the proposed guidelines. Moreover, we feel that mutagenesis and oncogenesis should be considered together as related rather than isolated phenomena. To these ends therefore, and in the interest of simplicity and practicality, the following core battery of tests selected from the proposed guidelines is recommended for mutagenicity screening of all pesticides.

1. A sophisticated microbiological test such as an enhanced Ames assay with appropriate dose response.
2. A mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma.
3. An in vivo cytogenetic assay.

In addition the Panel recommends that a multiple generation test which is part of the reproduction study (162.83-4), be modified to provide a statistically significant assay for dominant lethal mutations. The Panel also recommends that oncogenicity studies (162.83-2) be used in the overall evaluation of mutagenicity. Finally the Panel recommends that both the core battery of tests outlined in this report and the dominant lethal tests be performed on each pesticide. The results of these tests will provide the agency with the information necessary to estimate the mutagenic potential of pesticides".

The Scientific Advisory Panel is of the opinion that the stated requirement for a sophisticated microbiological test using benomyl has been met. In addition, the Panel believes that sufficient tests for in vivo cytogenetics have also been completed. It appears, however, that an appropriate mammalian cell point mutation assay has not been carried out using benomyl. Therefore, we recommend that a mammalian cell point mutation assay be performed using either a mouse lymphoma or a Chinese hamster embryo cell line or another equally well validated mammalian cell test for point mutation. If the results of this assay is negative, as has been the case for the microbiological test using Salmonella typhimurium mutants and the in vivo cytogenetics assays, we believe that no further mutation testing should be required and the mutation trigger should be considered to have been completely rebutted.

Spindle Effects: Recommendations for Testing

The Agency has submitted to the Scientific Advisory Panel a request for suggestions for methods for estimating risks in man from compounds such as benomyl which cause spindle effects. It is our opinion that the best methods for predicting adverse health effects in man from spindle poisons are the multigeneration reproductive test and the dominant lethal test. As noted in our previous recommendation on the proposed mutagenesis guidelines, we recommend that the multigeneration reproductive test be modified to provide a statistically significant assay for dominant lethal mutations. We believe that a multigeneration study modified to provide for an examination for dominant lethal mutations should be the primary means by which the agency estimates the potential risk to man from spindle poisons. If the multigeneration and dominant lethal studies are negative we recommend that cytogenetics be conducted on the sperm and bone marrow cells of an adequate number of the animals used in these or other studies. Consideration should also be given to the extension of the technique of amniocentesis and chromosome banding to the rodent model. In addition to the in vivo tests described above the Panel believes that it is desirable to have a reproducible and practical in vitro assay for spindle effects of pesticides, their contaminants, and breakdown products. A starting point may be an examination of metaphase arrest and growth inhibition in a mammalian cell line in culture (e.g. Chinese hamster embryo cells). In this regard, we urge the Agency to fund research with the aim of developing the amniocentesis rodent model and the in vitro test in mammalian cells for spindle effects of pesticides and pesticide products. Research should also be encouraged to evaluate the predictability of in vitro tubulin binding of spindle poisons.

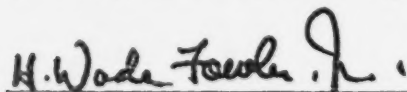
It is the opinion of the Scientific Advisory Panel that a threshold does exist for spindle effects from compounds such as colchicine and benomyl. This is based, in part, on the kinetics of the affinity of benomyl and colchicine for a tubulin binding site and the resulting inhibition of polymerization of tubulin to microtubules. This opinion is strengthened by the results of studies by Cox and Puck (1) in Chinese hamster cells in vitro using colcemid in which no appreciable changes in chromosomes were seen at 0.01 ug/ml. At 0.015 ug/ml nondisjunction appeared with tetraploidy occurring at 0.03 to 0.07 ug/ml. Similar results were obtained by Wilson et al using Vinca alkaloids in EHB cells in culture (2). In addition, studies by Seiler (3) with benomyl measuring micronucleated erythrocytes in mouse bone marrow following in vivo administration of benomyl also suggests there is a threshold for the spindle effects of this compound. The results of these studies (3) indicate the no effect level for benomyl for this measure of spindle effects in this mammalian species is between 400-500 mg/kg. Considering the level of exposure to benomyl of mixers and loaders and the exposure of the general population by way of the diet, the Scientific Advisory Panel is of the opinion that an adequate margin of safety exists for the potential spindle effects of benomyl in man.

In summary, the Panel believes that if negative results are obtained in an adequately conducted multigeneration reproductive test, in a dominant lethal test, and in the proposed in vivo cytogenetics assays, that this is adequate evidence that no adverse effects will occur in humans as a result of exposure to the spindle inhibitor in question. Since the results of a multigeneration reproductive test, the dominant lethal tests and the in vivo cytogenetics tests using benomyl have been negative the Panel is of the opinion that benomyl poses no significant risk as a spindle poison to human populations as a result of its current use in agriculture.

- (1) D.M. Cox and T. T. Puck, Cytogenetics 8: 158-169 (1969).
- (2) L. Wilson, K. Anderson and D. Chen, in Cell Motility, Cold Springs Harbor Symposium, D. Goldman, T. Pollard and J. Rosenbaum, eds., Cold Springs Harbor Press, New York, Vol. 3, 1051-1064 (1976).
- (3) J. D. Seiler, in Progress in Genetic Toxicology, D. Scott, B. A. Bridges and F. H. Sobels, eds., Elsevier, North Holland Biomedical Press, pp. 233-238 (1977).

FOR THE CHAIRMAN:

Certified as an accurate report of findings:



H. Wade Fowler, Jr., Ph.D.
Executive Secretary
FIFRA Scientific Advisory Panel

Date: November 29, 1979

RATIONALE REGARDING BENOMYL WARNING STATEMENT

We make these comments in relation to the broad issue of level of risk at which it is appropriate to list warning statements on pesticide labels. In the case of the fungicide benomyl, modification of the labeling on 5-pound or larger bags to include a warning statement indicating that birth defects or reduced sperm count might result from benomyl use is the issue in contention.

We encourage EPA to reconsider the proposed statement regarding reduced sperm count and birth defects in laboratory animals. We feel that warning statements should be reserved for pesticide uses that pose a demonstrated risk. Our assessment is that benomyl use does not constitute a hazard and that the warning statement would mitigate effectiveness and creditabilty of subsequent warning.

We make the above recommendation for the following reasons:

1. Benomyl is a non-volatile compound which is rapidly metabolized in mammalian systems to non-toxic metabolites, thus reducing risks from toxicity and accumulation in tissues (USDA/State/EPA Benomyl Assessment Report I).

October 1979

2. A study on dermal absorption presented by the registrant (DuPont) to the Scientific Advisory Panel (April, 1979) clearly indicates there is no exposure risk by a dermal route. The SAP commended DuPont for an excellent study. EPA has concluded, as a result, in PD 2/3 that dermal exposure does not cause an exposure risk.
3. EPA concluded in PD 2/3 that dietary intake is not of concern. The studies cited by EPA in PD 1 to support the presumption regarding teratogenic effects involved oral administration of benomyl with a no effect level of 62.5 mg/kg/day. Chronic feeding studies using rats and dogs indicated no effects on fertility (PD 1).
4. Evidence indicates therefore that any hazard to users is by inhalation of particulate matter. A cloth dust mask would significantly reduce this. EPA studies (PD 1) on the reduction of spermatogenic activity in rats and dogs using inhalation as route of administration indicate a no effect level of 7.5 mg/kg (in the more sensitive of the species -- rats). Theoretical calculations (Benomyl Assessment Report I) and actual data (study by registrant of inhalation exposure in a worse case situation indicating uptake of 0.016 mg/kg - submitted as public document September, 1979) indicate a very adequate margin of safety with regard to reduction of spermatogenesis and birth defects. Calculations in EPA PD 2/3 appear to be in error and should be recalculated.



DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON, D. C. 20250

OCT 9 1979



Honorable Douglas M. Costle (A-100)
Administrator
U. S. Environmental Protection Agency
Washington, D. C. 20460

Oct 22

JBN

Dear Mr. Costle:

The United States Department of Agriculture (USDA) is taking this opportunity to respond to the U. S. Environmental Protection Agency's (EPA) Preliminary Notice of Determination pursuant to 40 CFR 162.11(a)(5), concluding the Rebuttable Presumption Against Registration (RPAR) of Pesticide Products Containing Benomyl, and EPA's proposed intent to cancel and/or modify the terms and conditions of registration, pursuant to Section 6(b)(1) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (FIFRA).

We have interacted with EPA in developing biological, economic, and exposure information on benomyl. We are pleased to review and comment on the preliminary Notice of Determination and the accompanying position document.

We concur with the decision to continue registration of benomyl, with slight modification of the precautionary statements. The Department agrees that a fine woven cloth mask requirement would reduce exposure of aerial mixer/loaders to benomyl.

We encourage EPA to reconsider the proposed statement regarding reduced sperm count and birth defects in laboratory animals. We feel that warning statements should be reserved for pesticide uses that pose a demonstrated risk. Our assessment is that benomyl use does not constitute a hazard and that the warning statement would mitigate effectiveness and credibility of subsequent warnings. Rationale for this suggestion is enclosed.

We also have reservations regarding the water soluble packaging requirement for five pounds or larger quantities. Water soluble bags may reduce exposure of mixer/loaders to the chemical, but in exploring the feasibility of water soluble packaging, protection of these bags from the elements may be required. It is possible that losses may be incurred or human/environmental hazards created if the water soluble bags are inadvertently exposed to high humidity, dew, rain or damp storage. In addition, the five-pound bag size limitation may create disposal and/or overapplication problems when quantities other than five-pound increments must be used.

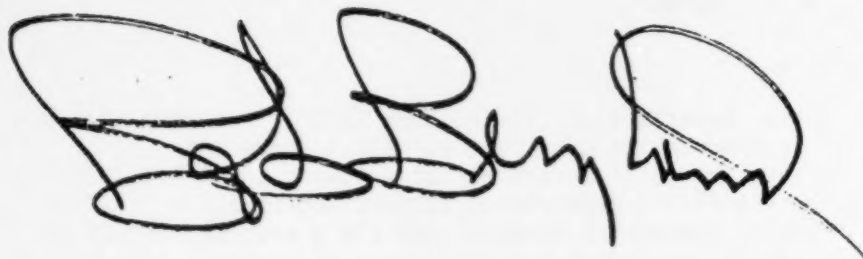
Andrew, 2/3

5(30000/23B)

*Hand-carried
from USA by J. J. J.*

We are dedicated to the mutual resolution of issues impacting the U. S. farm worker including health, crop production, and possible impacts on wildlife, nontarget organisms and other segments of the environment.

Sincerely,

A handwritten signature in black ink, appearing to read "Doug Costle". The signature is fluid and cursive, with the first name "Doug" and last name "Costle" clearly distinguishable.

Enclosure

ENVIRONMENTAL PROTECTION AGENCY

[OPP-30000/23C]

BENOMYL AND THIOPHANATE-METHYL

NOTICE OF DETERMINATION CONCLUDING THE REBUTTABLE PRESUMPTION AGAINST
REGISTRATION AND AVAILABILITY OF POSITION DOCUMENT

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of Determination; Notice of Availability.

SUMMARY: In December 1977, the Agency presumed against benomyl and thiophanate-methyl registrations because pesticide products containing these chemicals as active ingredients were shown to meet several of the Agency's risk criteria. This notice constitutes the final determination of the Agency concluding the RPAR proceedings for both benomyl and thiophanate-methyl. The Agency has determined that benomyl registrants must amend their labels to require use of protective equipment for persons who mix and load benomyl for aerial application. Benomyl registrants must also agree to conduct additional aquatic monitoring studies. Thiophanate-methyl registrants will not be required to take any action at this time.

DATE: Requests for a hearing must be received on or before (insert date 30 days after date of publication in the FEDERAL REGISTER) or within thirty (30) days from receipt of this notice, whichever occurs later.

ADDRESS: Requests for a hearing must be submitted to:

Hearing Clerk (A-110),
U.S. Environmental Protection Agency,
401 M St., SW.,
Washington, D.C. 20460.

Copies of the Decision Document are available upon request from:

Tom Miller,
Special Pesticide Review Division (TS-791),
Environmental Protection Agency,
Rm. 711-A, Crystal Mall #2,
1921 Jefferson Davis Highway,
Arlington, Virginia 22202.

FOR FURTHER INFORMATION CONTACT: Tom Miller (703-557-7420).

I. INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, requires the Environmental Protection Agency to review the risks and benefits of the uses of pesticides suspected of causing adverse effects to human health or the environment. In the FEDERAL REGISTER of December 6 and 7, 1977, (42 FR 61788 and 42 FR 61970) EPA issued Rebuttable Presumption Against Registration (RPAR) notices for pesticide products containing benomyl and thiophanate-methyl which are widely used fungicides. Their respective Position Document 1's (PD 1), published together with their RPAR notices, provided background and supporting data for the presumptions of risk cited in the RPAR notices. The rebuttable presumption for benomyl was based on evidence showing that it posed the following risks: acute toxicity to aquatic organisms, spermatogenic effects in test animals, mutagenic effects in various

organisms, teratogenic effects in test animals and effects to nontarget organisms. The rebuttable presumption for thiophanate-methyl was based on evidence showing that it posed a risk of mutagenicity and a risk to non-target organisms. The Agency also evaluated the oncogenic risks of both benomyl and thiophanate-methyl based upon studies received subsequent to issuance of both RPAR's. These studies showed that benomyl and the MBC metabolite of benomyl and thiophanate-methyl caused hepatocarcinogenicity in mice. In addition, both RPAR notices solicited information on the potential for benomyl, thiophanate-methyl, and their MBC metabolite to form carcinogenic N-nitrosamine compounds.

Benomyl is registered for use as a fungicide on various fruits, vegetables, nuts, field crops, flowers, ornamental plants and seed crops. Thiophanate-methyl is registered for use as a fungicide on various fruits, nuts, vegetables, turfgrass, flowers, ornamentals and shade trees.

Since the RPARs against both benomyl and thiophanate-methyl were issued, the Agency has reviewed rebuttal comments and data submissions and has conducted in-depth risk and benefit analyses. This notice announces the Agency's decision to continue registration for all uses of benomyl and thiophanate-methyl products as long as registrants comply with the terms and conditions of registration set forth in Unit III of this notice. This notice also announces the availability of the Position Document which details the Agency's analyses and rationale for the determination.

This notice is organized into four units. Unit I is this introduction. Unit II, entitled "Legal Background," is a general discussion of the regulatory framework within which those actions are taken. Unit III, entitled "Determinations and Initiation of Regulatory Action," sets forth the regulatory actions which the Agency is implementing concerning benomyl and thiophanate-

methyl, the bases for these determinations and a summary of the Agency's analysis of the risks and benefits of products containing these chemicals. Unit IV, entitled "Procedural Matters," discusses the procedures which will be followed in implementing the regulatory action which the Agency is announcing in this notice.

II. LEGAL BACKGROUND

In order to obtain a registration for a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended ("FIFRA"), a manufacturer must demonstrate that the pesticide satisfies the statutory standard for registration. That standard requires, among other things, that the pesticide perform its intended function without causing "unreasonable adverse effects on the environment," FIFRA section 3(c)(5). "Unreasonable adverse effects on the environment" is defined to mean "any unreasonable risk to man or the environment, taking into account the economic, social and environmental costs and benefits of the use of any pesticide," FIFRA section 2(bb)). In effect, this standard requires a finding that the benefits of each use of the pesticide exceed the risks of use, when the pesticide is used in accordance with commonly recognized practice and in compliance with the terms and conditions of registration. The burden of proving that a pesticide satisfies the registration standard is on the proponents of registration and continues as long as the registration remains in effect. Under section 6 of FIFRA, the Administrator may cancel the registration of a pesticide or modify the terms and conditions of registration whenever it is determined that the pesticide causes unreasonable adverse effects on the environment. The Agency created the RPAR process to facilitate the identification of pesticide uses

which may not satisfy the statutory standard for registration and to provide an informal procedure to gather and evaluate information about the risks and benefits of these uses.

The regulations governing the RPAR process are set forth at 40 CFR 162.11. That section provides that a rebuttable presumption shall arise if a pesticide meets or exceeds any of the risk criteria set out in the regulations. The Agency announces that an RPAR has arisen by publishing a notice of determination in the FEDERAL REGISTER. After an RPAR is issued, registrants and other interested persons are invited to review the data upon which the presumption is based and to submit data and information to rebut the presumption of risk by showing that the Agency's initial determination of risk was in error, or by showing that use of the pesticide is not likely to result in any significant exposure to human beings or the environment with regard to the adverse affects in question. In addition to submitting evidence to rebut the risk presumption, respondents may submit evidence as to whether the economic, social and environmental benefits of the use of the pesticide outweigh the risks of use.

The regulations require the Agency to conclude an RPAR by issuing a notice of determination. In that notice, the Agency is required to state and explain its position on the question of whether the risk presumption has been rebutted. If the Agency determines that the presumption is not rebutted, it will then consider information relating to the social, economic, and environmental costs and benefits which registrants and other interested persons submitted to the Agency, and any other benefits information known to the Agency. In determining whether the use of a pesticide poses risks which are greater than the benefits, the Agency considers possible changes to the terms

and conditions of registration which can reduce risks, and the impacts of such modifications on the benefits of the use. After weighing the risks and benefits of pesticide use, the Administrator may conclude the RPAR process by issuing a notice of intent to hold a hearing pursuant to section 6(b)(2) of FIFRA to determine whether the registrations for the use should be cancelled, denied or reclassified.

FIFRA section 6(b) requires the Agency to submit proposed notices pursuant to section 6 to the Secretary of Agriculture for comment and to provide the Secretary of Agriculture with an analysis of the impact of the proposed action on the agricultural economy. The Agency is required to publish any comments received from the Secretary and the Administrator's response to those comments with the notice of proposed action. During the pertinent time period, FIFRA section 25(d) also required the Administrator to submit section 6 notices to a Scientific Advisory Panel (SAP) for comment on the impact of the proposed action on health and the environment at the same time and under the same procedures as those described for review by the Secretary of Agriculture under section 6(b). After making any changes in the proposed action which are deemed appropriate as a result of the comments received, the Agency issues a notice containing its final determination of the regulatory actions (if any) which the Agency proposes to implement. Registrants and other people adversely affected by the Agency's actions can request a hearing to challenge the Agency's determination.

III. DETERMINATIONS AND INITIATION OF REGULATORY ACTION

The Agency has considered information on the risks associated with benomyl and thiophanate-methyl uses, including information submitted by registrants and other interested persons. The Agency has also considered information on

the social, economic and environmental benefits of the uses of these fungicides including benefits information submitted by registrants, the United States Department of Agriculture and other interested persons. The Agency's assessment of the risks and benefits of their uses, its conclusions and determinations whether their use poses unreasonable adverse effects on the environment, and its determination whether modifications in the terms or conditions of registration reduce risks sufficiently to eliminate any unreasonable adverse effects, are set forth in detail in the Position Document 4 on benomyl and thiophanate-methyl. That document is hereby adopted by the Agency as its statement of reasons for the determinations and actions announced in this notice, and as its analysis of the impacts of the proposed regulatory actions on the agricultural economy. For the reasons summarized below and developed in detail in the position document, the determinations of the Agency on benomyl and thiophanate-methyl are as follows.

A. DETERMINATIONS OF RISK

The Agency has made the following conclusions on the teratogenic, spermatogenic, mutagenic, oncogenic and aquatic risks of benomyl and the oncogenic and mutagenic risks posed by thiophanate-methyl.

1. Teratogenicity. The Agency has determined that benomyl has the potential to cause teratogenic effects. Teratogenic margins of safety (MOS's) from estimated worst case dietary exposures based upon a provisional no observed effect level (NOEL) of 30 mg/kg/day, derived from a gavage teratology study, range from 254 to 600,000 depending upon the commodity consumed. The Agency notes that teratogenic effects have not occurred in tests with benomyl employing dietary dosing. In general, the Agency considers the gavage route of dosing to be the method of choice for determinations of the NOEL for

teratogenic effects. For compounds such as benomyl, which are minimally absorbed and rapidly excreted from the system when taken in the diet, the Agency considers that risk extrapolation from gavage studies is appropriate because gavage studies more closely approximate human dietary exposures which peak periodically during ingestion of meals. The Agency recognizes, however, that dietary MOS's derived for chemicals in this way are conservative risk indicators.

Margins of safety for workers and users of benomyl are greater than 86 without respiratory protection and greater than 600 if a dust mask is used.

2. Spermatogenic effects. The Agency has determined that benomyl has the potential to cause spermatogenic effects. The NOEL for these effects is 7.5 mg/kg/day based on an inhalation study in rats. MOS's for spermatogenic effects from worst case dietary exposure estimates range from 64 to 150,000 depending upon the commodities consumed. MOS's for workers/users are greater than 21 without respiratory protection and greater than 150 if a dust mask is used.

3. Mutagenicity. The Agency considers the results from mammalian point mutation assays for benomyl and thiophanate-methyl to be uncertain and in need of confirmation. An existing mouse lymphoma study showed a weakly mutagenic response at a dose which killed about 80 percent of the test cells. Benomyl and MBC were not mutagenic in Chinese hamster ovary cells in vitro. Data suggest that positive results noted in certain point mutation studies could be due to contaminated test material. Results of point mutation tests on the MBC metabolite of both benomyl and thiophanate-methyl are negative for point mutagenicity. Data indicate that benomyl and MBC do not directly interact with DNA, although a contaminant in test material used in a number of the studies

might. An in vivo cytogenetics test with benomyl showed an increased frequency of polychromatic erythrocytes with micronuclei in mice given large doses of benomyl. However, the preponderance of data suggest that the observed results were more likely the result of spindle effects (microtubule inhibition during cell division) than direct chromosome damage. The agency has therefore determined that evidence is insufficient to conclude that benomyl and the MBC metabolite of both benomyl and thiophanate-methyl are point mutagens or that they directly interact with DNA or chromosome causing abnormalities.

Further, the Agency has determined that benomyl and/or the MBC metabolite of benomyl and thiophanate-methyl are spindle poisons capable of causing non-disjunction leading to aneuploidy or polyploidy, both chromosomal aberrations of concern. The impact of this effect to human health cannot be adequately assessed at this time.

4. Oncogenicity. The Agency has determined that both benomyl and the MBC metabolite of benomyl and thiophanate-methyl have been shown to be hepatocarcinogens in tests with mice. Based upon the most sensitive parameter for this effect, namely liver tumors from a study with MBC, the upper limit to the oncogenic risk to the general public via worst case dietary exposure was estimated to be 7×10^{-5} . This analysis assumes that food residues exist at tolerance levels and that 100 percent of crops with benomyl and thiophanate-methyl registrations are actually treated. When the percent of crop treated is considered, the risk estimate becomes about 3×10^{-5} . However, if average measured residues are used rather than tolerance levels, the upper bound of dietary oncogenic risk would be about 7×10^{-6} . For workers/users the upper bound of the oncogenic risk without respiratory protection ranges from 5×10^{-7} to 3×10^{-5} and with a dust mask from 7×10^{-7} to 5×10^{-6} .

The oncogenic risks presented by thiophanate-methyl are assumed to be similar, and in any event not greater than those presented by benomyl since both fungicides are similar in use and metabolism.

The worker exposure estimates used in generating the oncogenic risk estimate are based on the assumption that all of the inhaled product is absorbed from lung tissues and that this material is completely converted to MBC after being absorbed. In addition, maximum estimates of hours involved in work-related activities were used since precise data were not available. These factors tend to maximize the risk estimate. Evidence from a field study suggests that both the work-related exposure and dietary exposure could be an order of magnitude lower than the worst case calculations.

5. Risks to aquatic organisms. The Agency has determined that use of benomyl on rice presents potential risk to aquatic organisms. However, the Agency is not able to determine the level of risk to aquatic organisms from the use of benomyl in rice fields at this time. Additional field monitoring for benomyl residue levels in water will be required by the Agency.

B. DETERMINATIONS ON BENEFITS

The Agency has determined that benomyl is one of the more important fungicides for use on fruit crops. The Agency's brief review of benomyl's benefits for selected crops indicates that economic impacts from the unavailability of benomyl on all crops would range from more than \$26 million to more than \$52 million in a typical year for treatment costs alone. Additional impacts for lost production and higher consumer prices would also be expected; however, the Agency is not able to estimate the magnitude of these additional losses.

The benefits of thiophanate-methyl have not been quantified. This

fungicide is currently undergoing an expansion of its use registrations. Registrations for most new uses were granted within the last two years. Based on the fact that both products' fungicidal action is the result of their common metabolite, MBC, the Agency has assumed that both products are equally efficacious. However, the Agency does not now have comparative efficacy data to confirm that both products actually process equivalent efficacy.

The Agency has concluded that the benefits of use of thiophanate-methyl and benomyl are significant. These benefits could increase if increased market competition between both products leads to price reductions which would increase the overall usage of the products.

C. DETERMINATIONS ON UNREASONABLE ADVERSE EFFECTS

For reasons set forth in detail in the position document, the Agency has determined that the benefits of benomyl use exceed the risks of use if a dust mask is used when mixing and loading for aerial application. Therefore, the Agency has determined that the terms and conditions of benomyl-containing pesticide registrations and registration applications must be amended, as described below, or the use of benomyl-containing pesticides will "cause unreasonable adverse effects on the environment." In addition, for reasons set forth in the position document, the Agency has determined that the benefits of thiophanate-methyl use exceed the risks of such use as now labeled.

D. INITIATION OF REGULATORY ACTION

Based upon the determinations summarized above and developed in detail in the Position Documents 1, 2/3, and 4, the Agency has decided to continue registration of currently registered uses of benomyl products on the condition that certain changes are made in the terms and conditions of registration.

All benomyl registrations will be cancelled and all benomyl applications

for registration will be denied unless registrants and applicants amend the terms and conditions of their registration or request a hearing pursuant to sections 3 and 6 of FIFRA within 30 days from the date of publication of this notice in the FEDERAL REGISTER or from receipt of this notice, whichever occurs later.

This document shall constitute the Agency's notice of intention to cancel registrations for all benomyl-containing pesticide products which are labeled in a manner which permits aerial application unless registrants amend the terms and conditions of registration as follows:

Registrants must amend the product label for each registered product in five-pound packages or larger, which do not prohibit aerial application, to read as follows:

Harmful if inhaled. Wear a cloth or disposable paper dust mask during handling and mixing.

E. OTHER DETERMINATIONS - TESTING REQUIREMENTS

Under section 3(c)(2)(B) of FIFRA, the Agency will notify registrants of benomyl-containing pesticides that they must agree to conduct, jointly or separately, testing to maintain current registrations. The requirement for these data will be independent of the Agency's RPAR determination as set forth in this notice to modify the terms of registration. A monitoring study to determine levels of benomyl residues in aquatic sites near rice fields will be required. This test must be submitted within the time frame specified in the Agency's 3(c)(2)(B) letter regarding this requirement which is being sent to benomyl registrants. The Agency will review this study with the intent of resolving the scientific uncertainties associated with the current aquatic risk assessment.

F. AGENCY ANALYSES OF COMMENTS

The Agency received comments on the August 3, 1979, Notice of Determination and the PD 2/3 from the Scientific Advisory Panel (SAP) and the U.S. Department of Agriculture (USDA).

1. Comments of the SAP. The comments of the SAP on the Notice of Determination Concluding the Rebuttable Presumption Against Registration of Pesticides Containing Benomyl and Thiophanate-methyl are presented below in their entirety.

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA)

SCIENTIFIC ADVISORY PANEL

Review of Preliminary Notices to

Conclude Rebuttable Presumptions Against Registration
(RPARS) of products containing benomyl and thiophanate-methyl

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel has completed review of plans by the Environmental Protection Agency for initiation of regulatory action on benomyl and thiophanate-methyl pesticide products under the provisions of section 6(b)(1) of FIFRA, as amended. The review was completed during open meetings in Arlington, Virginia, during the periods October 9-10, 1979, and November 29, 1979.

Maximum public participation was encouraged for the review. FEDERAL REGISTER notices were published on September 21, 1979, November 14, 1979, and November 27,

1979. In addition telephonic calls and special mailings were sent to the general public who had previously expressed an interest in activities of the Panel.

Written and oral statements were received from representatives of E. I. DuPont de Nemours and Company and Pennwalt Corporation in respect to proposed regulatory action on benomyl and thiophanate-methyl, respectively. In consideration of all matters brought out during the meeting and careful review of all documents submitted by the Agency and other parties, the Panel unanimously submits the following report:

Panel Position on Thiophanate-methyl

The Scientific Advisory Panel concurs with the Agency's position to terminate the RPAR against thiophanate-methyl. In regard to the option under consideration by the Agency for additional gene mutation tests for MBC, a metabolite of thiophanate-methyl, the Scientific Advisory Panel offers the following comment:

In May 1978, the FIFRA Scientific Advisory Panel issued the following statement relative to the mutagenesis subsection of subpart F, Hazard Evaluation: Human and Domestic Animals, of the Proposed Guidelines for Registering Pesticides in the United States:

"The Scientific Advisory Panel strongly endorses the need for mutagenic assays as a survey for potential environmental hazards and endorses the

principles of the proposed guidelines. Moreover, we feel that mutagenesis and oncogenesis should be considered together as related rather than isolated phenomena. To these ends, therefore, and in the interest of simplicity and practicality, the following core battery of tests selected from the proposed guidelines is recommended for mutagenicity screening of all pesticides.

1. A sophisticated microbiological test such as pan enhanced Ames assay with appropriate dose response.

2. A mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma.

3. An in vivo cytogenetics assay.

In addition, the Panel recommends that a multiple generation test which is part of the reproductive study (162.83-4) be modified to provide a statistically significant assay for dominant lethal mutations. The Panel also recommends that oncogenicity studies (162.83-2) be used in the overall evaluation of mutagenicity. Finally, the Panel recommends that both the core battery of tests outlined in this report and the dominant lethal test be performed on each pesticide. The results of these tests will provide the Agency with the information necessary to estimate the mutagenic potential of pesticides".

In evaluating the data available on the mutagenicity of MBC, the Scientific Advisory Panel has also evaluated mutagenicity studies with benomyl since metabolism studies have shown that at least 80 percent of benomyl is metabolized to MBC in vivo. On examination of the mutagenicity tests that have been conducted using MBC, benomyl, and thiophanate-methyl, the Panel is of the opinion that the requirement for a sophisticated microbiological test has been met. In addition, the Panel is of the opinion that a suitable in vivo cytogenetics assay has been completed. However, the Panel does not believe that an appropriate mammalian cell point-mutation assay has been performed. Therefore, we recommend that a mammalian cell point mutation assay be conducted using MBC and a mouse lymphoma or Chinese hamster embryo cell line or any other equally well validated test for point mutations in mammalian cells. If the results of this test are negative, the Scientific Advisory Panel is of the opinion that the mutagenic potential of thiophanate-methyl be considered to have been completely rebutted and no further tests should be required. In regard to the potential for spindle effects from human exposure to thiophanate-methyl, the Scientific Advisory Panel offers the following conclusions: A negative result has been obtained in a multigeneration reproductive study and a dominant lethal study using thiophanate-methyl. The Scientific Advisory Panel is of the opinion that these

two tests and an appropriate in vivo cytogenetics test constitute an adequate measure for adverse health effects in man from spindle poisons. Having met the proposed requirements the Scientific Advisory Panel is of the opinion that there is no significant risk from spindle inhibition in man as a result of exposure to thiophanate-methyl in its normal use in agriculture.

PANEL POSITION ON BENOMYL

In regard to the specific issues and questions posed by the Agency to the Panel regarding the benomyl RPAR, the Scientific Advisory Panel offers the following responses:

Questions from EPA Concerning Its Regulatory Decision.

Question 1. Based on the data available at this time do you agree with the agency's conclusion of low risk from the spindle effects associated with use of benomyl?

Answer: Yes (see Appendix I).

Question 2. Would you suggest a method of estimating the mutagenic risk for spindle effects?

Answer: See Appendix I.

Question 3. We proposed to request gene mutation tests in (1) Drosophila, (2) mammalian somatic cells in culture, and (3) an appropriate eukaryotic microorganism. In your opinion are these tests adequate?

Answer: See Appendix 1.

Question 4. During the April 1979 Scientific Advisory Panel meeting we discussed the DuPont dermal absorption

study. Would you comment on this study and the agency's conclusion that the dermal component of the total body dose is extremely low.

Answer: We have examined the data from the study conducted by DuPont on dermal absorption of 2-¹⁴C-benomyl through rat skin. We concur with the agency's position that it would not be possible to obtain a level of benomyl in the blood through dermal absorption which would pose a significant risk.

Panel Comments on Benomyl Regulatory Decision

The EPA has proposed the continued registration of benomyl for all uses, but with an amendment of the terms and conditions of registration. These amendments of terms and conditions of registration entail the requirement for (1) additional mutation studies, (2) the requirement for water soluble packaging for benomyl sold in units of 5 pounds or larger, (3) that a cloth mask be required during mixing and loading for aerial application if the product is not packaged in water soluble bags, (4) that a monitoring study of the fate of benomyl in the environment be required for continued registration for rice uses and, (5) that label changes will be required including a precautionary statement relative to reproductive hazards.

Mutation Studies (1)

Concerning the requirement for additional mutation studies see Appendix 1.

Water Soluble Packaging and Respiratory Protection (2) and (3)

The FIFRA Scientific Advisory Panel supports the requirement that a protective mask be worn during the mixing, loading and transfer operations for aerial application of benomyl. However, the Panel recommends the use of commercial disposable paper masks, in addition to the cloth mask specified in the decision document be allowed if, in the opinion of the agency, they provide sufficient protection against respiratory exposure to benomyl during these operations. With the recommendation for use of respiratory protection in mixing, loading and transfer operations involving benomyl, the Scientific Advisory Panel believes that the requirement for water soluble packaging for units of 5 pounds or larger should not be required.

Monitoring Studies (4)

The Panel supports the requirement for additional monitoring studies on benomyl for rice use. These studies will allow the EPA to make a more accurate determination of the potential risks to aquatic organisms of the use of benomyl on rice.

Label Changes (5)

The Scientific Advisory Panel does not concur in the suggested precautionary warning statement to be included on the label for benomyl. The basis for the Panel's non-concurrence is that if this type of labeling is required for

benomyl it should also be required for all other pesticide products which cause adverse health effects in man. This, of course, would include almost all pesticide products currently in use. The Panel might favorably consider specific health effects warning statements on pesticide labels were this requirement uniformly applied to all pesticide products. However, we are of the opinion that the selective requirement for this kind of warning label on benomyl is not appropriate in view of past regulatory decisions that have been made relative to other pesticide products that have undergone an examination through the RPAR process.

Additional Comments

The no effect level for teratogenesis from benomyl administered by gavage to rats has not yet been established. Two studies, one by the Environmental Protection Agency and another by DuPont are currently underway in which the Scientific Advisory Panel has an interest. Any conclusions by the Panel favoring continued use of benomyl are contingent upon a no effect level for reproductive toxicology being established in these studies. Furthermore, the level at which no effect is seen should provide a satisfactory margin of safety relative to the exposure levels which have been calculated from dietary intake and exposure during mixing, loading, transfer or application of benomyl. It should be noted that dietary

studies of the teratogenic properties of both benomyl and the breakdown product MBC in rats have been carried out and the results of these studies fail to show an effect at dose levels of 400 mg/kg/day. Teratogenic studies in rabbits at approximately 125 mg/kg/day in the diet also showed no evidence of compound related teratogenicity. Therefore, it is the opinion of the Scientific Advisory Panel that any no effect level established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in these dietary studies. Except as noted above, the Panel concurs with the Agency's position for continued registration of benomyl for all uses.

APPENDIX I

Mutation Studies

The Scientific Advisory Panel offers the following comment in regard to the agency's request that additional gene mutation tests using benomyl be carried out in Drosophila mammalian somatic cells in culture, and in an appropriate eukaryotic microorganism.

On May 31, 1973, the FIFRA Scientific Advisory Panel issued the following statement relative to the mutagenesis subsection of subpart F, Hazard Evaluation: Human and Domestic Animals, of the Proposed Guidelines for Registering Pesticides in the United States:

"The Scientific Advisory Panel strongly endorses the need

for mutagenic assays as a survey for potential environmental hazards and endorses the principles of the proposed guidelines. Moreover, we feel that mutagenesis and oncogenesis should be considered together as related rather than isolated phenomena. To these ends therefore, and in the interest of simplicity and practicality, the following core battery of tests selected from the proposed guidelines is recommended for mutagenicity screening of all pesticides.

1. A sophisticated microbiological test such as an enhanced Ames assay with appropriate dose response.
2. A mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma.
3. An in vivo cytogenetic assay.

In addition the Panel recommends that a multiple generation test which is part of the reproduction study (162.83-4), be modified to provide a statistically significant assay for dominant lethal mutations. The Panel also recommends that oncogenicity studies (162.83-2) be used in the overall evaluation of mutagenicity. Finally the Panel recommends that both the core battery of tests outlined in this report and the dominant lethal tests be performed on each pesticide. The results of these tests will provided the agency with the information necessary to estimate the mutagenic potential of pesticides".

The Scientific Advisory Panel is of the opinion that the stated requirement for a sophisticated microbiological

test using benomyl has been met. In addition, the Panel believes that sufficient tests for in vivo cytogenetics have also been completed. It appears, however, that an appropriate mammalian cell point mutation assay has not been carried out using benomyl. Therefore, we recommend that a mammalian cell point mutation assay be performed using either a mouse lymphoma or a Chinese hamster embryo cell line or another equally well validated mammalian cell test for point mutation. If the results of this assay is negative, as has been the case for the microbiological test using Salmonella typhimurium mutants and the in vivo cytogenetics assays, we believe that no further mutation testing should be required and the mutation trigger should be considered to have been completely rebutted.

Spindle Effects: Recommendations for Testing

The agency has submitted to the Scientific Advisory Panel a request for suggestions for methods for estimating risks in man from compounds such as benomyl which cause spindle effects. It is our opinion that the best methods for predicting adverse health effects in man from spindle poisons are the multigeneration reproductive test and the dominant lethal test. As noted in our previous recommendation on the proposed mutagenesis guidelines, we recommend that the multigeneration reproductive test be modified to provide

a statistically significant assay for dominant lethal mutations. We believe that a multigeneration study modified to provide for an examination for dominant lethal mutations should be the primary means by which the agency estimates the potential risk to man from spindle poisons. If the multigeneration and dominant lethal studies are negative we recommend that cytogenetics be conducted on the sperm and bone marrow cells of an adequate number of the animals used in these or other studies. Consideration should also be given to the extension of the technique of amniocentesis and chromosome banding to the rodent model. In addition to the in vivo tests described above the Panel believes that it is desirable to have a reproducible and practical in vitro assay for spindle effects of pesticides, their contaminants, and breakdown products. A starting point may be an examination of metaphase arrest and growth inhibition in a mammalian cell line in culture (e.g. Chinese hamster embryo cells). In this regard, we urge the Agency to fund research with the aim of developing the amniocentesis rodent model and the in vitro test in mammalian cells for spindle effects of pesticides and pesticide products. Research should also be encouraged to evaluate the predictability of in vitro tubulin binding of spindle poisons. It is the opinion of the Scientific Advisory Panel that

a threshold does exist for spindle effects from compounds such as colchicine and benomyl. This is based, in part, on the kinetics of the affinity of benomyl and colchicine for a tubulin binding site and the resulting inhibition of polymerization of tubulin to microtubules. This opinion is strengthened by the results of studies by Cox and Puck (1) in Chinese hamster cells in vitro using colcemid in which no appreciable changes in chromosomes were seen at 0.01 ug/ml. At 0.015 ug/ml nondisjunction appeared with tetraploidy occurring at 0.03 to 0.07 ug/ml. Similar results were obtained by Wilson et al using Vinca alkaloids in EHB cells in culture (2). In addition, studies by Seiler (3) with benomyl measuring micronucleated erythrocytes in mouse bone marrow following in vivo administration of benomyl also suggests there is a threshold for the spindle effects of this compound. The results of these studies (3) indicate the no effect level for benomyl for this measure of spindle effects in this mammalian species is between 400-500 mg/kg. Considering the level of exposure to benomyl of mixers and loaders and the exposure of the general population by way of the diet, the Scientific Advisory Panel is of the opinion that an adequate margin of safety exists for the potential spindle effects of benomyl in man.

In summary, the Panel believes that if negative results are obtained in an adequately conducted multigeneration reproductive test, in a dominant lethal test, and in the proposed in vivo cytogenetics assays, that this is adequate evidence that no adverse effects will occur in humans as a result of exposure to the spindle inhibitor in question. Since the results of a multigeneration reproductive test, the dominant lethal tests and the in vivo cytogenetics tests using benomyl have been negative the Panel is of the opinion that benomyl poses no significant risk as a spindle poison to human populations as a result of its current use in agriculture.

(1) D. M. Cox and T. T. Puck, Cytogenetics 8: 158-169 (1969).

(2) L. Wilson, K. Anderson and D. Chen, in Cell Motility, Cold Springs Harbor Symposium, D. Goldman, T. Pollard and J. Rosenbaum, eds., Cold Springs Harbor Press, New York, Vol. 3, 1051-1064 (1976).

(3) J. D. Seiler, in Progress in Genetic Toxicology, D. Scott, B. A. Bridges and F. H. Sobels, eds., Elsevier, North Holland Biomedical Press, pp. 233-238 (1977).

FOR THE CHAIRMAN:

Certified as an accurate report of findings:

H. Wade Fowler, Jr., Ph.D.

Executive Secretary

FIFRA Scientific Advisory Panel

Date: November 29, 1979 121<

2. Comments of the USDA. The comments of the United States Department Agriculture (USDA) on the Notice of Determination Concluding the RPAR of Pesticides Containing Benomyl are presented below in their entirety.

Honorable Douglas M. Costle (A-100)

Administrator

U. S. Environmental Protection Agency

Washington, D. C. 20460

Dear Mr. Costle:

The United States Department of Agriculture (USDA) is taking this opportunity to respond to the U. S.

Environmental Protection Agency's (EPA) Preliminary Notice of Determination pursuant to 40 CFR

162.11(a)(5), concluding the Rebuttable Presumption

Against Registration (RPAR) of Pesticide Products

Containing Benomyl, and EPA's proposed intent to cancel and/or modify the terms and conditions of registration, pursuant to section 6(b)(1) of the Federal Insecticide, Fungicide, and Rodenticide Act, as Amended (FIFRA).

We have interacted with EPA in developing biological, economic, and exposure information on benomyl. We are pleased to review and comment on the preliminary Notice of Determination and the accompanying position document. We concur with the decision to continue registration of benomyl, with slight modification of the precautionary statements. The Department agrees that a fine woven cloth mask requirement would reduce exposure of aerial

mixer/loaders to benomyl.

We encourage EPA to reconsider the proposed statement regarding reduced sperm count and birth defects in laboratory animals. We feel that warning statements should be reserved for pesticide uses that pose a demonstrated risk. Our assessment is that benomyl use does not constitute a hazard and that the warning statement would mitigate effectiveness and credibility of subsequent warnings. Rationale for this suggestion is enclosed.

We also have reservations regarding the water soluble packaging requirement for five pounds or larger quantities. Water soluble bags may reduce exposure of mixer/loaders to the chemical, but in exploring the feasibility of water soluble packaging, protection of these bags from the elements may be required. It is possible that losses may be incurred or human/environmental hazards created if the water soluble bags are inadvertently exposed to high humidity, dew, rain or damp storage. In addition, the five-pound bag size limitation may create disposal and/or overapplication problems when quantities other than five-pound increments must be used.

We are dedicated to the mutual resolution of issues

impacting the U.S. farm worker including health, crop production, and possible impacts on wildlife, nontarget organisms and other segments of the environment.

Sincerely,

Bob Bergland

Enclosure

RATIONALE REGARDING BENOMYL WARNING STATEMENT

We make these comments in relation to the broad issue of level of risk at which it is appropriate to list warning statements on pesticide labels. In the case of the fungicide benomyl, modification of the labeling on 5-pound or larger bags to include a warning statement indicating that birth defects or reduced sperm count might result from benomyl use is the issue in contention.

We encourage EPA to reconsider the proposed statement regarding reduced sperm count and birth defects in laboratory animals. We feel that warning statements should be reserved for pesticide uses that pose a demonstrated risk. Our assessment is that benomyl use does not constitute a hazard and that the warning statement would mitigate effectiveness and creditability of subsequent warning.

We make the above recommendation for the following reasons:

1. Benomyl is a non-volatile compound which is rapidly metabolized in mammalian systems to non-toxic metabolites, thus reducing risks from toxicity and accumulation in tissues (USDA/State/EPA Benomyl Assessment Report I).

2. A study on dermal absorption presented by the registrant (DuPont) to the Scientific Advisory Panel (April, 1979) clearly indicates there is no exposure risk by a dermal route. The SAP commended DuPont for an excellent study. EPA has concluded, as a result, in PD 2/3 that dermal exposure does not cause an exposure risk.

3. EPA concluded in PD 2/3 that dietary intake is not of concern. The studies cited by EPA in PD 1 to support the presumption regarding teratogenic effects involved oral administration of benomyl with a no effect level of 62.5 mg/kg/day. Chronic feeding studies using rats and dogs indicated no effects on fertility (PD 1).

4. Evidence indicates therefor that any hazard to users is by inhalation of particulate matter. A cloth dust mask would significantly reduce this. EPA studies (PD1) on the reduction of spermatogenic activity in rats and dogs using inhalation as route of administration indicate a no effect level of 7.5 mg/kg (in the more sensitive of the species — rats). Theoretical calculations (Benomyl

Assessment Report I) and actual data (study by registrant of inhalation exposure in a worse case situation indicating uptake of 0.016 mg/kg - submitted as public document September, 1979) indicate a very adequate margin of safety with regard to reduction of spermatogenesis and birth defects. Calculations in EPA PD 2/3 appear to be in error and should be recalculated.

3. Response to comments of the SAP.—(a) Point Mutagenicity and Direct Chromosomal Effects. With regard to the SAP's conclusions on the existing mutagenicity assays on benomyl, MBC, and thiophanate-methyl, the Agency considers the results from mammalian point mutation assays with benomyl to be uncertain and in need of confirmation. The existing mouse lymphoma study showed a weakly mutagenic response (mutation frequency 2 to 3 times that in the control culture) at a dose which killed about 80 percent of the test cells. Benomyl and MBC did not cause mutations in Chinese hamster ovary cells in vitro. Data suggest that positive results noted in certain point mutation studies could be due to contaminated test material. Study results on the MBC metabolite of benomyl and thiophanate-methyl are negative for point mutagenicity. Data indicate that benomyl and MBC do not directly interact with DNA, although their contaminant might do so. Dominant lethality was not demonstrated in an existing test with benomyl. An in vivo cytogenetics test with benomyl showed an increased frequency of polychromatic erythrocytes with micronuclei in mice given up to 1,000 mg/kg doses of benomyl. However, the preponderance of data suggest that the observed results were more likely the

result of spindle effects (microtubule inhibition during cell division) than direct chromosome damage.

(b) Spindle effects testing. The SAP indicated that the best methods for predicting adverse health effects in man from spindle poisons were the multigeneration reproductive test and the dominant lethal test. The multigeneration study modified in this way was recommended as the primary means by which the Agency should estimate the potential risk to man from spindle poisons. In the SAP's view, if results in this test system were negative, cytogenetics should be conducted on the sperm and bone marrow cells of an adequate number of the animals used in this and other studies. SAP also urged the Agency to fund research with the aim of developing the amniocentesis rodent model and the in vivo test in mammalian cells for spindle effects, as well as research on the evaluation of the predictability of in vivo tubulin binding of spindle poisons.

The Agency differs with the SAP in that it does not consider the in vivo tests recommended by the Panel (multigeneration reproductive, dominant lethal and cytogenetics) sufficiently sensitive to assess adequately risk from spindle inhibitors. The Agency does agree, however, with the desirability for the development of sensitive in vivo assays for spindle effects, as well as the funding of research on the predictive value of tubulin binding. The Agency cannot suggest at this time an alternative set of tests, since none of the assay systems currently proposed for use in screening programs can unequivocally detect chemicals producing spindle effects significantly relevant to man, such as aneuploidy by nondisjunction or any other mechanisms. The

following is a portion of a summary statement by OPP staff scientists delineating the position of the Agency in this regard:

The Scientific Advisory Panel (SAP) submitted to the Office of Pesticide Programs (OPP) its evaluation of chemicals which may interfere with the cell-division spindle (benomyl, thiophanate-methyl and trifluralin) on November 30, 1979 (Appendix A). The SAP is willing to accept data from a few test systems which are presently available (reproduction and dominant lethal tests plus in vivo cytogenetics) to evaluate safety in regard to spindle effects. Using this scheme the Panel concluded that adequate testing has been done for benomyl in this regard.

On the one hand, OPP agrees with the Panel that nothing in the review of all the information available on these chemicals, including subchronic toxicity and reproduction studies, indicated significant adverse effects from this toxicity. On the other hand, OPP does not think these systems have the capability to assess fully the degree of hazard from spindle inhibition.

Basically, OPP staff is of the opinion that there is a lack of information bearing on the sensitivity and adequacy of various tests to evaluate risks from spindle inhibition and, more generally, inhibition of microtubular protein polymerization.

In addition to in vivo tests, the Panel also suggested research funding for the development of in vivo assays for

spindle effects of pesticides, contaminants and breakdown products (such as examining metaphase arrest in mammalian cells), as well as approaches to evaluate the predictability of in vivo tubulin binding of spindle poisons.

The OPP staff agrees with the SAP as to the need for further investigation on the usefulness of various systems for evaluating risks. To this end, the Agency plans to identify outside scientists who can help us delineate a meaningful research program to fill some of the information gaps and to summarize possible means of risk assessment.

The problem of assessing risks is heightened when one considers on a broader scale the development of schemes for chemicals which inhibit microtubular polymerization. In this case, one is concerned not only with spindle effects but also all other effects produced by chemicals acting via the molecular mechanism of microtubular inhibition. Since these inhibitors affect all microtubule-related processes, including cell shape, cell movement, intracellular molecular movement, and cellular secretion, many functions may be affected by these 'cellular poisons.' Given these effects, we must decide what are the appropriate toxicological endpoints of concern, how to evaluate them and, lastly, how to evaluate risks. Because of the magnitude of the problem we have decided to start with spindle effects and to enlarge the scope of problems over time.

(c) Spindle effects threshold. SAP also stated that it believed a threshold existed for spindle effects from compounds such as benomyl. Considering the level of exposure to benomyl (mixer/loaders and consumers via the diet) SAP was of the opinion that an adequate margin of safety existed for the potential spindle effects of benomyl in man. In addition SAP was of the opinion that there was no significant risk from spindle inhibition in man as a result of exposure to thiophanate-methyl in its normal use in agriculture.

The SAP based its opinion on a threshold for benomyl on the kinetics of the affinity for the same tubulin binding site of colchicine and benomyl, and the resulting inhibition of polymerization of tubulin to microtubules. The Panel's opinion was strengthened by studies with mammalian cells using the other well-known anti-mitotic agents, colcemid and the vinca alkaloids, as well as by a mouse micronucleus test with benomyl all suggesting a threshold for spindle effects.

The Agency disagrees with the opinion expressed by the SAP. A threshold for spindle effects and nondisjunction cannot be unequivocally demonstrated by the studies cited for either the antimitotic alkaloids, nor for benomyl. The in vitro studies were conducted with cell lines (CHO, EHB) displaying inconstant chromosome numbers; unsynchronized cell populations were treated; and no control cell clones were isolated for comparison to the treated isolates. The dose range between the concentration of agent needed to block movement of single chromosomes and that needed to cause complete mitotic arrest is extremely narrow (e.g., in the case of colcemid, 0.015 ug/ml and 0.03 ug/ml). Any reference to a mechanism involving what relative percentage of tubulin must be bound to account for selective inhibition of polymerization leading to "non-disjunction" is tenuous. The above data also show that only

3 percent of bound cellular tubulin appears to be critical to block the entire spindle thus leading to polyploidy. Cellular tubulin is involved as well in cellular processes other than the mitotic spindle. There is no evidence for thresholds involved in these processes.

The in vivo study cited was actually performed with methyl benzimidazole carbamate (MBC), the principle active metabolite of benomyl. The author reported no adverse effect (no increase in micronuclei) of MBC given orally to mice at a (single) dose of 50 mg/kg corresponding to a blood level of 8 ug/ml (the solubility limit of the compound). Further, the inference of a threshold for spindle effects (microtubular) was drawn by the investigator from his in vitro experiments with isolated pig brain preparations, in which $5 \times 10^{-5}M$ (approximately 10 ug/ml) was the minimal effect level (25 percent inhibition of tubulin association), while $10^{-4}M$ (12 ug/ml) gave maximum effect (100 percent inhibition). Finally, the micronucleated erythrocytes which are counted as the end-effect in this test are not exclusively the product of spindle inhibition; micronuclei also occur following treatment with chromosome breakers. No effect levels one fifth to one tenth those for micronuclei have been reported with benomyl/MBC for reproductive effects in rats at 7.5 mg/kg, only a portion of which may be attributable to the chemical's effects on tubulin. Hence, the circulating threshold may conceivably be <8 ug/ml for these effects.

(d) Teratogenic effects. The SAP agreed that a NOEL for benomyl administered by gavage to rats had not yet been established. Conclusions by the SAP favoring continued use of benomyl were contingent upon a NOEL for reproductive toxicology being established in studies then underway by DuPont and EPA. Furthermore, this level should provide a satisfactory MOS relative to dietary and worker exposure levels. SAP noted that dietary studies of the

teratogenic properties of both benomyl and MBC in rats had been carried out and failed to show an effect at dose levels of 400 mg/kg/day. Teratogenic studies in rabbits at approximately 125 mg/kg/day in the diet also showed no evidence of compound related teratogenicity. Therefore, the SAP stated that any NOEL established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in dietary studies.

The Agency is aware that the results obtained by the two methods may differ. However, the Agency notes that both gavage and dietary administration have produced toxicologically significant results in animal tests.

The Agency considers gavage dosing to be more relevant than dietary dosing for extrapolating human risk from chemicals like benomyl which have short half-lives. Even so, the Agency considers that gavage may be a more conservative indicator of this risk and in interpreting MOS's, the Agency has taken this into consideration. The Agency estimates indicate margins of safety greater than 86 without a dust mask and greater than 600 with a dust mask for non-dietary exposure. Margins of safety ranging from 254 to 600,000 were estimated for maximum single serving dietary doses of benomyl.

(e) Comments relating to the regulatory decision. The SAP supported the requirement that a protective mask be worn during the mixing, loading and transfer operations for aerial application of benomyl. However, the SAP recommended the use of commercial disposable paper masks be allowed if, in the opinion of the Agency, they provide sufficient protection against exposure to benomyl during these operations.

The Agency agrees with the SAP that the use of a disposable paper mask would provide sufficient protection. Therefore, the requirement for a dust

mask can be satisfied by use of either a finely woven cloth mask or disposable paper mask.

The SAP indicated that water-soluble packaging should not be required since the Agency was requiring use of a cloth mask to reduce exposure to aerial application mixer/loaders.

DuPont has provided information to the Agency regarding the use of water-soluble films for packaging of benomyl in Florida. They concluded that such packaging was impractical and uneconomical. In particular, problems developed with regard to maintaining the product's guaranteed strength in this packaging medium.

The Agency agrees with the comment that both water-soluble packaging and a mask need not be used at the same time. The Agency is aware that technical problems, such as preventing the reaction of benomyl with the film, must be overcome before water-soluble bags can be used. Therefore, the Agency agrees that water-soluble packaging for benomyl is not technically feasible at this time.

The SAP did not concur in the suggested precautionary warning statement to be included on the label for benomyl. The basis for their non-concurrence was that if this type of labeling were required for benomyl it should also be required for all other pesticide products which cause adverse health effects in man. The SAP indicated they might favorably consider specific health effects warning statements on pesticide labels were this requirement uniformly applied to all pesticide products. However, this selective requirement for a warning statement on benomyl was not considered appropriate in view of past regulatory decisions made relative to other pesticide products that have undergone the RPAR process.

The Agency has withdrawn the proposal for additional warning labeling for benomyl since the risk analysis contained in the position document indicates that risks from most exposure situations are low when compared to the benefits and for the highest risk situation, mixer/loaders for aerial application, these risks will be reduced to an acceptable level in comparison with the benefits if a dust mask is used. Therefore, additional warning labeling is no longer considered necessary.

The SAP supported the requirement for additional monitoring studies on benomyl for the rice use to allow the Agency to make a more accurate determination of the potential risks to aquatic organisms from this use.

4. Responses to comments of the USDA.—(a) Comments on the regulatory decision. The USDA concurred with the Agency decision to continue registration of benomyl and agreed that a finely woven cloth mask requirement would reduce exposure of aerial mixer/loaders to benomyl.

The USDA had reservations regarding the water-soluble packaging requirement for five-pound or larger quantities. They stated that protection of water-soluble bags from the elements might be required. Benomyl stored in such bags might sustain losses or cause human or environmental hazards if they were exposed to high humidity, dew, rain or damp storage. They also felt that the five-pound bag size limitation might create disposal and/or overapplication problems when quantities other than five-pound increments were needed.

The Agency disagrees with USDA that greater care would be needed in protecting benomyl packaged in water-soluble bags than is presently required in protecting benomyl from the elements. The current DuPont benomyl label cautions against permitting 'Benlate' to become wet during storage. The

Agency did not suggest that the water-soluble bags should contain five pounds of benomyl. Pesticides currently sold in water-soluble bags (including experimental sales of benomyl) are usually packaged in one-half pound bags (i.e. ten bags per five pounds).

USDA urged the Agency to reconsider the proposed label statement regarding reduced sperm count and birth defects in laboratory animals. USDA felt that warning statements should be reserved for demonstrated risk situations. USDA did not consider benomyl to present a hazard and, therefore, contended that such a statement would mitigate the effectiveness and credibility of subsequent warnings.

The Agency has withdrawn the proposal for additional warning labeling for benomyl since the risk analysis contained in the position document indicates that risks from most exposure situations are low when compared to the benefits and for the highest risk situation, mixer/loaders for aerial application, these risks will be reduced to an acceptable level in comparison with the benefits if a dust mask is used. Therefore, such additional warning labeling is no longer considered necessary.

IV. PROCEDURAL MATTERS

This notice initiates actions to cancel registrations and deny applications for registration of benomyl products for all uses permitting aerial application unless the terms and conditions of registration are modified to comply with the requirements of this notice. Under sections 6(b)(1) and 3(c)(6) of FIFRA, applicants and registrants and certain other interested or adversely affected persons may request a hearing on the cancellation and denial actions that this notice initiates. Unless the necessary steps to make these changes are taken within the statutory 30-day period or unless a hearing is

properly requested to contest the proposed action, the cancellation or denial actions will become final at the end of the statutory period.

This unit of the notice explains how registrants and applicants may seek to make any necessary corrections and modify the terms and conditions of registration and how registrants, and other adversely affected or interested persons may request a hearing on the cancellation and denial actions set forth in this notice.

A. PROCEDURES FOR AMENDING THE TERMS AND CONDITIONS OF REGISTRATION

Registrants affected by the cancellation actions described above in unit IID may avoid cancellation by amending the terms and conditions of registration within the 30-day period. This must be done by filing an application for an amended registration with the Agency. A statement must appear under the precautionary statements section of the labeling of products packaged in 5-pound or larger packages which do not prohibit aerial application as follows:

Harmful if inhaled. Wear a cloth or disposable paper dust mask during handling and mixing.

The application for an amended registration must be submitted to:

Henry Jacoby,
Product Manager (PM) 21,
Registration Division (TS-767C),
Office of Pesticide Programs,
Environmental Protection Agency,
Rm. 227, CM #2,
1921 Jefferson Davis Highway,
Arlington, VA 22202,
(703-557-1900).

B. PROCEDURE FOR REQUESTING A HEARING

Registrants adversely affected by the cancellation actions described above may request a hearing on such actions within thirty (30) days of receipt of this notice or within thirty (30) days from publication of this notice in the FEDERAL REGISTER, whichever occurs later. Any other person adversely affected by the cancellation actions described above may request a hearing within thirty (30) days from publication of this notice in the FEDERAL REGISTER.

Applicants for registration affected by the denial action described above may request a hearing on such actions within thirty (30) days of receipt of this notice or within thirty (30) days from publication of this notice in the FEDERAL REGISTER, whichever occurs later. Other interested persons may, with the concurrence of the applicant, request a hearing during the time period available to the applicant.

All registrants and other affected parties who request a hearing must file the request in accordance with the procedures established by FIFRA and the Agency's Rules of Practice Governing Hearings (40 CFR Part 164). These procedures require among other things that:

- (1) All requests must identify the specific registration(s) by registration numbers and the specific use(s) for which a hearing is requested.
- (2) All requests must be accompanied by objections that are specific for each use of the identified pesticide products for which a hearing is requested.
- (3) All requests must be received by the hearing clerk within the applicable thirty-(30) day period. Failure to comply with these requirements will automatically result in denial of the request for a hearing.

Requests for hearings must be submitted to:

Hearing Clerk: (A-110),

U.S. Environmental Protection Agency,

401 M St., SW.,

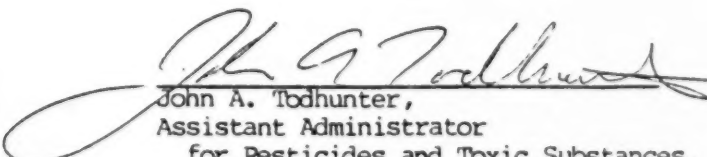
Washington, D.C. 20460.

C. CONSEQUENCE OF FILING OR FAILING TO FILE A HEARING REQUEST

1. Consequence of filing a timely and effective hearing request. If a hearing on any action initiated by this notice is requested in a timely and effective manner, the hearing will be governed by the Agency's Rules of Practice for hearings under FIFRA section 6 (40 CFR Part 164). In the event of a hearing, each cancellation and denial action subject to the hearing will not become effective except pursuant to an order of the Administrator at the conclusion of the hearing.

2. Consequences of failure to file in a timely and effective manner.
If no hearing concerning the conditional cancellation or denial of registration of a specific use of a specific pesticide product containing benomyl has been requested by the end of the applicable thirty-day period, registration of that benomyl product will be cancelled or the denial will become effective unless the registrant or applicant amends its label within the statutory period to include the requirements specified in this notice for a given use.

Dated: Oct 1, 1982


John A. Todhunter,
Assistant Administrator
for Pesticides and Toxic Substances.